As correctly indicated in the Office Action Summary, claims 9, 10 and 12 are pending in the application and are under consideration.

Rejection over Blache and Nevia

Claims 9, 10 and 12 have been newly rejected under 35 U.S.C. § 103 as allegedly being unpatentable over U.S. Patent Number 5,523,322 ("Blache") in view of Neiva et al., Brazilian Journal of Medical and Biological Research, 30:599-604, 1997 ("Nevia"). The rejection is traversed.

The prior art fails to establish a proper prima facie case of obviousness. To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. § 2143.

The cited references fail to establish a prima facie case of obviousness when properly considered in the context of the state of the art at the time the present application was filed, because the state of the art as a whole would not have suggested the proposed combination or provided the person of ordinary skill in the art with a reasonable expectation of success.

The totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness. *In re Hedges,* 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986); Manual of Patent Examination Procedure § 2145. It is impermissible to first ascertain factually what applicants did and then view the prior art in such a manner as to select from the random facts of that art only those which may be modified and then utilized to reconstruct applicant's invention from such prior art. *See, e.g., Interconnect Planning Corp.*

v. Feil, 227 U.S.P.Q. 543, 550 (Fed. Cir. 1985); *see also, In re Shuman*, 150 U.S.P.Q. 54, 57 (C.C.P.A 1966).

In asserting this rejection, the Office has proposed a combination of teachings that would not have been accepted by a person of ordinary skill in the art at the time that the present application was filed. The Office alleges that Blache teaches a method of inhibiting blood platelet aggregation with compounds of the kind recited in the present claims. The Office acknowledges that Blache does not teach a method of treating a disease recited in the present claims. The Office alleges that Nevia teaches that that platelet aggregation occurs in Alzheimers patients. The Office wrongly alleges that this teaching would make it obvious to treat Alzheimer's disease using the compounds taught by Blache.

By the time that the Blache application was first filed in 1993 it was established that there was no link between the platelet aggregation which could cause vascular problems and the development of symptoms of Alzheimer's disease. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) was already in place (since 1986) and its purpose was to develop brief, standardized and reliable procedures for the evaluation and diagnosis of patients with Alzheimer's Disease and other dementias of the elderly. The first results of CERAD and the various teams of the Consortium displayed clearly that "*...in neuropathologic study of patients clinically diagnosed as having Alzheimer's disease, ... only two of 150 cases showed a purely vascular basis of dementia*" See, Mirra et al., Neurology, 41:479-86, 1991(attached as Exhibit A). In considering the only two cases of vascular dementia that had been found among Alzheimer's Patients, it must be remembered that simply having a vascular involvement in a dementia patient does not automatically mean a blood-platelet aggregation problem.

In accordance with the CERAD findings and the state of the art in 1993 with regards to Alzheimer's disease, the inventors of Blache did not consider the possibility of treating Alzheimer's with their compounds for inhibiting blood platelet aggregation. They focused their disclosure on a method of inhibiting blood platelet aggregation "*for the treatment or prevention of arterial thrombotic complications (cerebral vascular injury, myocardial infarction) or venous thrombotic complications (phlebitis) and any vascular injury connected with artherosclerosis (in particular cerebral), in the treatment and the prevention of ischemic injuries, in the treatment of blood platelet disorders*" (Blache col. 1, lines 64-67, col. 2 lines 1-3).

Contrary to the allegation of the Office, in 1997 the authors of Nevia did not, in fact, show that "*...platelet aggregation occurs in Alzheimer's Disease...*" Rather, Nevia states that "*Aluminum intoxication is thought to play a major role in the development of Alzheimer's disease and in certain pathologic manifestations arising from long-term hemodialysis*" (abstract, 1st sentence) and that "*aluminium overload is frequently associated with neurological disorders such as Alzheimer's encephalopathy, amyotrophic lateral sclerosis and aging*" (Introduction, 1st paragraph). Nevia reports no measurement of platelet aggregation in patients with Alzheimer's disease. The conclusion of the authors of Nevia is that aluminum "*induces oxidative stress in platelets, stimulating lipoxygenase activity in these cells and promoting their aggregation*". This reflects the former belief in the field that Alzheimer's disease was caused by aluminum exposure, but that hypothesis had been completely excluded by the time of the present invention. *See, e.g.* Mirra et al. 1991 whole article (Exhibit A *supra*), *and* Cummings et al. Neurology, 51:S2-S17, 1998 (attached as Exhibit B).

In 1998, it was known that "*...less than 1% of pathologically-defined Alzheimer's disease cases show major vascular involvement*" *See*, Smith M.A., *J Chem Neuroanatomy*, 16:35-41, 1998 (attached as Exhibit C). As stated above, a vascular involvement does not automatically mean an implication of blood platelet aggregation. The review by Cummings et (Exhibit B, *supra*) states that "C*urrently available Alzheimer's disease-specific therapies are of two types: symptomatic approaches based on enhancement of cholinergic function, and neuroprotective approaches utilizing antioxidant agents*" Cummings, at S12, 2nd column, 2nd paragraph.

In 1999 the accepted wisdom with regards to Alzheimer's disease was that "*oxidative imbalance and stress are key elements in the pathogenesis of Alzheimer's disease*" *See*, Smith et al., *J Histochem Cytochem*, 46:731-35, 1998 (attached as Exhibit D). In the CNS, peroxynitrite (product of the reaction between nitric oxide and superoxide) which is a strong oxidizing and nitrating is known to be responsible for neuronal damages. Peroxynitrite can be generated by microglial cells activated by pro-inflammatory cytokines or β-amyloid peptide and by neurons in three different situations: hyperactivity of glutamate transmission, mitochondrial dysfunction and depletion of L-arginine or tetrahydrobiopterin. *See*, Torreilles et al., *Brain Research Reviews*, 30:153-63, 1999 abstract (attached as Exhibit E). By 1999, it was known and proved that aluminum intoxication could not explain the physiopathology of Alzheimer's disease even if epidemiological evidence suggests that elevated levels of aluminum in brain tissue may be linked to progression of Alzheimer's disease. By 1999, no published article showed any direct link between platelet aggregation problems and Alzheimer's disease.

Thus, the state of the art in 1999 would have regarded the hypothesis attributed to Nevia by the Office as contrary to the accepted wisdom and there would not have been any

motivation or suggestion to arrive at the present invention by combining Nevia with Blache as the Office has alleged. Moreover, if a person of ordinary skill in the art were motivated to try a treatment based upon the Office's reading of unproven speculation in Nevia, it would not have been obvious to choose the compounds of the present invention. It must be noted that in 1999, a great many platelet aggregation inhibitors were known, so that following the Office's logic in combining Blache in view of Nevia, one might have tried to treat Alzheimer's disease with any of the myriad of platelet aggregation inhibitors that were known. But that was never done and would not have produced the results that are produced by the present invention.

Because in 1999 it was accepted wisdom in the art that Alzheimer's disease was not caused by platelet aggregation problems but due to many other causes among which senile plaques in the extra cellular space and neurofibrillary tangles within vulnerable neurons, it would not have been obvious for one skilled in the art to implement a method for the treatment of Alzheimer's disease using a compound able to inhibit blood-platelet aggregation.

The compounds of the invention acts on the hyperactivity of glutamate transmission. In 1999, the discovery of the present invention was novel and far from obvious. The antiglutamatergic properties of the compounds of the invention were surprisingly discovered by Maurice Israël and his team and in consideration of these surprising properties, they decided that the compounds of the invention could become disease modifying drugs for conditions and diseases were the glutamate transmission is affected.

For at least the foregoing reasons, the rejection is improper and should be withdrawn.

Rejection over Blache and Neu

Claims 9, 10 and 12 stand rejected under 35 U.S.C. § 103 as allegedly unpatentable over U.S. Patent Number 5,523,322 ("Blache") in view of Neu et al., *Acta Neurol. Scandinav.* 66:497-504, 1982 ("Neu"). The rejection is traversed.

Blache is discussed above. Neu is alleged to teach that platelet aggregation occurs in Multiple Sclerosis (MS) patients. The Office erroneously concludes that this teaching would make it obvious to treat MS using the compounds taught by Blache.

The authors of Neu worked on Putnam's "*vascular hypothesis for multiple sclerosis*". Their postulate is that studies were made on platelet adhesiveness but almost nothing was published on platelet aggregation in MS patients. They performed measurements on 30 MS patients. Their conclusion is that "*...MS patients showed a (significantly) increased ADP- and serotonin-induced platelet aggregation.*" This is not a measurement of platelet aggregation in a native biological system (spontaneous aggregation) but in an induced system (via ADP or serotonin). Regarding spontaneous aggregation, the authors asserted that "*An increased tendency to spontaneous aggregation of the blood platelets of MS patients could also be demonstrated*". They merely hypothesized that spontaneous aggregation could be demonstrated but they don't show it! Their conclusion is that "*Neither the results of our own investigations provide a conclusive answer to the question of whether the platelet alterations in MS patients are epiphenomena of multiple sclerosis, or whether they are independent, pathogenetically relevant phenomena*" Neu at 503, §(1).

A review by McKhann in 1982 gives a good overview of the state of the art in MS at the time Neu was published. McKhann, Ann. Rev. Neurosci., 5:219-39, 1982 (attached as Exhibit F)("*The pathological analysis indicates a selective disease process with loss of myelin and oligodendroglia ... there remains the possibility that there is more generalized*

involvement of white matter or blood vessels.") Neu was published in 1982 and as of 1999, there was no confirmation of Neu's hypothesis. No direct link was established between platelet aggregation problems and the development of MS even if there might be some vascular involvement in symptoms of MS.

In the late 80's, two theories were popular in the scientific and medical fields: the infection cause (probably viral) and the autoimmune cause. It the early 90's it was established that MS is a demyelinating disease of the white matter of the CNS in which myelin and other related compounds are destroyed. The autoimmune theory was favored because of several new studies and data. Merrill et al., *West J. Med.*, 156:639-46, 1992 (attached as Exhibit G). By the time that that the first Blache application was filed in 1993 it was already established that there was no direct link between the platelet aggregation which could cause vascular problems and the development of symptoms of MS. According to those results and the state of the art in 1993, the inventors of Blache focused their invention on a method of inhibiting blood platelet aggregation "*for the treatment or prevention of arterial thrombotic complications (cerebral vascular injury, myocardial infarction) or venous thrombotic complications (phlebitis) and any vascular injury connected with artherosclerosis (in particular cerebral), in the treatment and the prevention of ischemic injuries, in the treatment of blood platelet disorders*" Blache col. 1, lines 64-67, col. 2 lines 1-3.

In 1993, Blache would have had the benefit of Neu's 1982 publication of their hypothesis. The fact that Blache et al. does not even mention MS shows that the teachings of Neu would have been given no consideration by persons of ordinary skill in the art in possession of a compound capable of inhibiting platelet aggregation. In 1993, if it was obvious for one skilled in the art that the compounds of the invention described in Blache were potential drug candidates for the treatment of MS, Blache et al. would not have missed a

chance to claim patent rights on such a promising market. The Blache patent application was filed by Roussel-UCLAF which is now a part of the Sanofi Aventis Group, an organization skilled in the art of searching and developing treatments for neurological diseases among which MS.

In 1996, in an article entitled "Multiple Sclerosis Treatment" by Andersson et al. *West J Med*, 1996 (attached as Exhibit H), the patients were treated for each symptom with: glucocorticosteroids and corticotrophin during acute exacerbations, bacoflen and physic exercises for spasticity, anticholinergic agents for bladder symptoms, dietary adjustments for bowel symptoms, amantadine hydrochloride, pemoline or fluoxetine hydrochloride for fatigue, antidepressant for depression (eg. amitriptyline, fluoxetine), tricyclic antidepressant (eg. amitriptyline) for pain syndromes, clonazepam for tremor and ataxia. In 1999 according to a better knowledge of the development of the pathology the drugs believed to act on the course of the disease were interferon beta. See, Larner et al., British Medical Journal, 319-362-6, 1999 at 363 1st column (attached as Exhibit I) and anti-inflammatory drugs See, Parish et al., Immunology and Cell Biology, 76:104, 1998 abstract only (attached as Exhibit J).

In line with the state of the art in 1999, no drugs approved to treat MS were directed to regulation of platelet aggregation or intended to act on any vascular problem. Rudick, Arch. Neurol., 56:1079-84, 1999 (attached as Exhibit K) in its article entitled "*Disease-Modifying drugs for relapsing-remitting MS and future directions for MS therapeutics*" gives an overview of the state of the art with regards to disease modifying therapies for MS back in 1999. Table 1 (page 1080) lists the drugs approved by the FDA.

The compounds of the invention act on the hyperactivity of glutamate transmission. The antiglutamatergic properties of the compounds of the invention were surprisingly discovered by Maurice Israël and his team and is consideration of these surprising properties,

they decided that the compounds of the invention could become disease modifying drugs for all conditions and diseases were the glutamate transmission is affected.

Furthermore, if the logic of the Office in the proposed combination were followed by a person of ordinary skill in the art, they might have tried to treat MS with any of the myriad known platelet aggregation inhibitors. However, doing so would not have produced the results of the presently claimed invention, because platelet aggregation is not a critical factor in MS. Even if the cause(s) of MS remains unknown, it was established in the art before 1999 that platelet aggregation problems can't explain the complex physiopathology of MS. MS is thought to be an autoimmune pathology which involves a genetic susceptibility but is not directly inherited. Consequently it would not have been obvious for one skilled in the art to implement a method for the treatment of MS using a compound able to inhibit blood-platelet aggregation.

When the totality of the state of the art s given due consideration, it is clear that the presently claimed invention would not have been obvious in view of the cited art. There would have been no genuine suggestion or reasonable expectation of success in performing the claimed method. Therefore, the rejection should be withdrawn.

Rejection over Blache and Lechner

Claims 9, 10 and 12 stand rejected under 35 U.S.C. § 103 as allegedly unpatentable over U.S. Patent Number 5,523,322 ("Blache") in view of Lechner et al., Weiner medizinische Wochenschrift, 136:387-91, 1986 ("Lechner"). The rejection is traversed.

Blache is discussed above. Lechner is alleged to teach that platelet aggregation occurs in Parkinsonism patients. The Office erroneously concludes that this teaching would make it obvious to treat Parkinson's Disease (PD) using the compounds taught by Blache.

In the late 60's and early 70's high dose oral levodopa therapy was established as the most effective therapy ever found for PD. The authors of Lechner report results of a study on a particular group of PD patients with a vascular risk. The term "Lechner-Ott-Syndrom" is not found in the general literature on PD, only Lechner himself published few articles directed to the "Lechner-Ott-Syndrom." In this article Lechner states that "*...zwischen 85 und 90% des anfallenden Krankengutes dem idiopathischen Parkinson zugeordnet zu werden...*" Lechner at page 387, §2. This means that almost 90% of the PD population is diagnosed with idiopathic PD. Idiopathic PD is the most common form of Parkinsonism, and is not attributed to vascular causes. Even the maximum of about 10% that can be diagnosed with ischemic PD, also called vascular parkinsonism, does not mean that these patients have platelet aggregation problems. The commonly accepted clinical features of vascular parkinsonism are different from those of idiopathic PD.

Lechner preceded Blache by several years, so that Blache would have had the benefit of Lechner's paper at the time the Blached application was filed. In 1993, when the earliest Blache application was filed, it was already known that PD was caused by the loss of dopaminergic neurons in the substantia nigra pars compacta. The patients were still treated with levodopa and other treatment strategies (potentiation of levodopa effects, prevention or delay neuronal cell death) were studied. None of the treatments studied at the time of the Blacu was directed to inhibition of platelet aggregation because PD is not a vascular problem. See, e.g. Marsden, Lancet, April 21, 1990, pp. 948-52 (attached as Exhibit L). In the early 90's, accepted wisdom in the art was that "*Research into Parkinson's disease in the next decade will centre on improvements in neuroprotective treatment to prevent or slow the rate of progression of the disease; methods of protection against free radical damage; the role of excitatory amino acid antagonists, and specific methods of delivery of such agents to the*

brain; and early diagnosis for the most effective use of neuroprotective agents." *Id.* at 951, 2nd paragraph.

In 1996 Montastruc et al. stated that "*Although levodopa remains the 'gold standard' in the treatment of the disease...several neuroprotective drugs are now in development in experimental research...*" Montastruc et al., *Drugs and Aging*, 9:169-84, 1996 at 169 (attached as Exhibit M). It is still believed that a neuroprotective approach could be a good way of treatment of PD. An article by Poewe et al. demonstrated that the contemporary studies in the late 1990's were directed to the "*...development of neuroprotective strategies that would modify the progression of disease..*" Poewe et al., *Ann Neurol.*, 44:S1-S9, 1998 at §1 (attached as Exhibit N). The neuroprotective agents expected to give results are "*...antioxidants including vitamine C, tocopherol and deprenyl, as well as antiglutamate agents including amantadine, dopamine agonists and neuronal growth factors*" Id. at S5, §2. There is also a good clinical review of the predicted developments in PD from 1999 stating that "*clinical trials of new drugs with neuroprotective and neurorescue properties are in progress.*" Schapira et al., British Medical Journal, 318:311-14, 1999 at 311 (attached as Exhibit O)

At the time of the present invention, it was accepted wisdom that PD should be treated with neuroprotective agents. It would have been contrary to the accepted wisdom to proceed as the Office has proposed. Even if the logic of the Office in the proposed combination were followed by a person of ordinary skill in the art, they might have tried to treat PD with any of the myriad known platelet aggregation inhibitors. However, doing so would not have produced the results of the presently claimed invention, because platelet aggregation is not a cause of PD.

The compound of the invention acts on the hyperactivity of glutamate transmission. In 1999 The antiglutamatergic properties of the compounds of the invention were surprisingly discovered by Maurice Israël and his team and is consideration of these surprising properties, they decided that the compounds of the invention could become disease modifying drugs for all conditions and diseases were the glutamate transmission is affected.

Because PD is not due to platelet aggregation problems but to many other causes among which the degeneration of dopamine-producing nerve cells in the brain, specifically in the substantia nigra and the locus coeruleus (PD patients have lost 80% or more of their dopamine-producing cells by the time symptoms appear), it would not have been obvious for one skilled in the art to implement a method for the treatment of PD using a compound disclosed as able inhibit blood-platelet aggregation.

When the totality of the state of the art is given due consideration, it is clear that the presently claimed invention would not have been obvious in view of the cited art. There would have been no genuine suggestion or reasonable expectation of success in performing the claimed method. Therefore, the rejection should be withdrawn.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

The Director is hereby authorized to charge any appropriate fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: November 6, 2006

By: _____
Christopher L. North
Registration No. 50,433

P.O. Box 1404
Alexandria, VA 22313-1404
703 836 6620

EXHIBIT A

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD).

Part II. Standardization of the neuropathologic assessment of Alzheimer's disease

S.S. Mirra, MD; A. Heyman, MD; D. McKeel, MD; S.M. Sumi, MD; B.J. Crain, MD, PhD; L.M. Brownlee, BChE, MD; F.S. Vogel, MD; J.P. Hughes, MS; G. van Belle, PhD; L. Berg, MD; and participating CERAD neuropathologists*

Article abstract—The Neuropathology Task Force of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) has developed a practical and standardized neuropathology protocol for the postmortem assessment of dementia and control subjects. The protocol provides neuropathologic definitions of such terms as "definite Alzheimer's disease" (AD), "probable AD," "possible AD," and "normal brain" to indicate levels of diagnostic certainty, reduce subjective interpretation, and assure common language. To pretest the protocol, neuropathologists from 15 participating centers entered information on autopsy brains from 142 demented patients clinically diagnosed as probable AD and on eight nondemented patients. Eighty-four percent of the dementia cases fulfilled CERAD neuropathologic criteria for definite AD. As increasingly large numbers of prospectively studied dementia and control subjects are autopsied, the CERAD neuropathology protocol will help to refine diagnostic criteria, assess overlapping pathology, and lead to a better understanding of early subclinical changes of AD and normal aging.

NEUROLOGY 1991;41:479-486

The Consortium to Establish a Registry for Alzheimer's Disease (CERAD), a multicenter study, has developed brief, comprehensive, and reliable clinical and neuropsychological batteries for assessment of patients clinically diagnosed as having probable Alzheimer's disease (AD) as Morris et al[1] recently reported. This current paper reports the subsequent development of a practical standardized protocol for the neuropathologic evaluation of autopsy brains of demented and control subjects. A task force of neuropathologists from nine university medical centers in the United States was formed to achieve the following immediate objectives: (1) to create a neuropathology protocol consisting of an illustrated guidebook and data entry form,[2] (2) to facilitate the entry of neuropathologic findings into the CERAD information system to be linked with clinical information on demented and cognitively normal subjects, and (3) to establish a mechanism for the continual refinement of the protocol to reflect new technical and scientific developments.

The long-range goals of the protocol are to produce more accurate and reliable neuropathologic criteria for AD, to determine the neuropathologic spectrum of AD, and to establish the types and frequency of other disorders coexisting with AD or occurring alone. The protocol is not intended to characterize each case definitively. It is designed instead to provide a simple, easily understood, and uniform approach that will indicate levels of diagnostic certainty, reduce subjective interpretation, and assure common language. Consequently, it is particularly valuable as a framework for the documentation of neuropathologic data on "borderline" cases, eg, demented subjects with few neocortical plaques or tangles, or, conversely, nondemented cases with neuropathologic evidence of AD.

To pretest the protocol, neuropathologists from 15 CERAD centers submitted neuropathology data from 142 consecutive brain autopsies on patients clinically diagnosed at their institutions as having probable AD[3] and on eight subjects who had no evidence of cognitive impairment or neurologic disease. This report describes the CERAD neuropathology protocol and presents the findings from these 150 autopsies.

* See Acknowledgments on page 485.

From the Veterans Administration Medical Center and Emory University School of Medicine (Drs. Mirra and Brownlee), Atlanta, GA; Duke University Medical Center (Drs. Heyman and Crain), Durham, NC; Washington University (Drs. McKeel and Berg), St. Louis, MO; and the University of Washington (Drs. Sumi and Vogel, J.P. Hughes, and Dr. van Belle), Seattle, WA.

Supported by NIA #AG06790. Dr. Mirra's work is also supported by Veterans Administration Merit Award #5747-4.

Received September 5, 1990. Accepted for publication in final form October 1, 1990.

Address correspondence and reprint requests to Dr. Suzanne S. Mirra, VA Medical Center (113), Department of Pathology and Laboratory Medicine, 1670 Clairmont Road, Decatur, GA 30033.

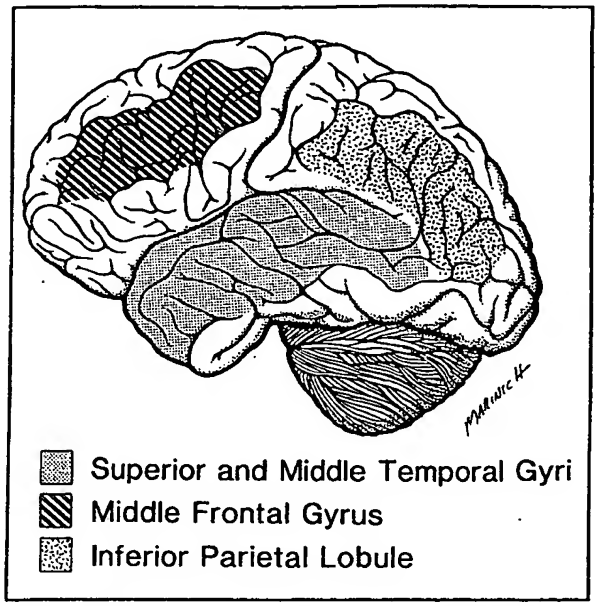


Figure 1. This diagram of the lateral surface of the brain illustrates the areas of neocortex from which recommended neocortical sections are taken.

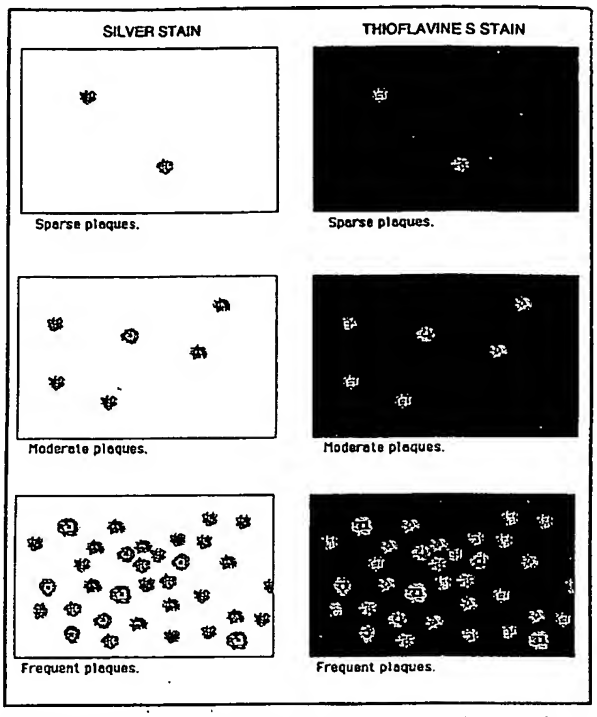


Figure 2. Senile plaques (neuritic) per 100× microscopic field. This cartoon provides a guide to semiquantitative assessment of plaque density per square millimeter.

Methods. *Description of the CERAD neuropathology protocol. Gross findings.* The data entry form documents availability of brain and spinal cord tissue, brain weight, and the presence of any gross abnormalities in brain, spinal cord, or meninges. The degree of regional neocortical atrophy and ventricular enlargement, if any, is rated semiquantitatively (none, mild, moderate, severe). The presence or absence of atrophy of the hippocampus and entorhinal cortex as well as pallor of the substantia nigra and locus ceruleus are also recorded. The cerebral blood vessels are examined grossly for atherosclerosis or significant obstruction and aneurysms or other anomalies. The number, size, frequency, distribution, and laterality of lacunar and large infarcts as well as hemorrhages are also recorded.

Microscopic preparations. A minimum of five anatomic regions are designated for microscopic study. Requisite sections include middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, hippocampus and entorhinal cortex, and midbrain, including the substantia nigra. Guidelines for the neocortical regions from which the sections are taken are provided (figure 1). Most of the centers participating in this CERAD study routinely sample additional areas of the brain as part of their evaluations.

The neuropathology guidebook recommends that paraffin-embedded sections be cut at a thickness of 6 to 8 micrometers. In addition to hematoxylin-eosin or other general stains, a sensitive silver stain such as the modified Bielschowsky method is recommended for the detection of senile plaques and neurofibrillary tangles. (Use of the Bodian preparation is not recommended.) The fluorescent thioflavine S preparation viewed under ultraviolet light is accepted as an alternative stain for plaques and tangles as well as for cerebral amyloid. The Congo red stain also may be used for evaluating cerebral amyloid. More conventional or traditional histopathologic methods were deliberately recommended as these are used in virtually all neuropathology laboratories. Many laboratories, of course, supplement these techniques with immunocytochemical procedures that may enhance detection of pathologic changes, such as amyloid deposition using beta/A4 antibodies and Lewy bodies using antiubiquitin or other antibodies.

CERAD diagnostic neuropathologic criteria for AD. These diagnostic criteria are based upon the semiquantitative assessment of neocortical senile plaques of the neuritic type, ie, those with thickened silver-positive neurites. Presently, the protocol does not require specification of the number or proportion of diffuse plaques, ie, plaques without discernible abnormal neurites or fibrillar amyloid (also known as "very primitive," "amorphous," or "amyloid"); both neuritic and diffuse plaques label positively with the beta-A4 amyloid protein antibody.[4-10] The pathogenesis and clinical significance of different plaque types remain controversial. Some neuropathologists believe that diffuse plaques are more commonly encountered in nondemented elderly individuals whereas neuritic plaques are more characteristic of AD[4]; others observe that diffuse plaques are the commonest type encountered in AD.[10] Moreover, the morphologic distinction between plaque type is not always clearcut, and less sensitive staining methods may not detect diffuse plaques.[11] Future modifications of the neuropathology protocol will reflect the evolving understanding of the importance of plaque subtype.

The CERAD neuropathologic diagnosis is derived from a three-step process:

Step 1. In order to encourage participation, standardize observations, and avoid time-consuming counts, neuropathologists are first asked to make semiquantitative assessments of the frequency of senile plaques and neurofibrillary tangles in the neocortex in areas of maximum density. The overall degree of vascular amyloid deposition and the proportion of plaques containing amyloid cores are also noted. Micrographs and cartoon illustrations representing examples of mild, moderate, and severe plaque frequencies are provided as guides (figure 2).

Table 1. Age-related plaque scores*

Age of pt at death (yrs)	Frequency of plaques†			
	None	Sparse	Moderate	Frequent
<50	0	C	C	C
50-75	0	B	C	C
>75	0	A	B	C

* An age-related plaque score is determined using patient's age along with plaque frequency in the most heavily affected neocortical section.
† Based on section of frontal, temporal, or parietal cortex with maximum involvement.

For purpose of this protocol, the letter circled corresponds to the following assessment:

0 = NO histologic evidence of Alzheimer's disease.
A = Histologic findings are UNCERTAIN evidence of Alzheimer's disease.
B = Histologic findings SUGGEST the diagnosis of Alzheimer's disease.
C = Histologic findings INDICATE the diagnosis of Alzheimer's disease.

Table 2. Neuropathology diagnosis: Diagnostic criteria for Alzheimer's disease

Normal (with respect to AD or other dementing processes)	a	No histologic evidence of Alzheimer's disease (0 score), *and* no clinical history of dementia, *and* absence of other neuropathologic lesions likely to cause dementia
(choose one)	b	An "A" age-related plaque score *and* no clinical history of dementia
	c	A history of dementia *and* absence of any neuropathologic lesions likely to cause dementia
Definite		"C" age-related plaque score, *and* clinical history of dementia, *and* presence or absence of other neuropathologic lesions likely to cause dementia
CERAD NP probable*		"B" age-related plaque score, *and* clinical history of dementia, *and* presence or absence of other neuropathologic disorders likely to cause dementia
CERAD NP possible*		"A" age-related plaque score, *and* clinical history of dementia, *and* presence or absence of other neuropathologic lesions that could cause dementia
(choose one)	a	
	b	"B" or "C" age-related plaque score *and* absence of clinical manifestations of dementia

* Not to be confused with the NINCDS-ADRDA clinical criteria (McKhann et al, *Neurology* 1984;34:939-944).

The age-related plaque score is integrated with the presence or absence of a clinical history of dementia to arrive at a diagnostic level of certainty with regard to Alzheimer's disease.

Step 2. An age-related plaque score is then determined by combining the age of the patient at death and the semiquantitative measure of plaques in the most severely affected region of the neocortex (table 1).

Step 3. This score is then integrated with clinical information regarding the presence or absence of dementia to determine the level of certainty of the diagnosis of AD (table 2). The terms "definite," "probable," and "possible AD" refer here only to the neuropathologic diagnoses and should not to be confused with the NINCDS/ADRDA criteria for the clinical diagnosis of AD.[3]

Evaluation of other pathologic findings. In addition to the gross examination for evidence of cerebrovascular disease described earlier, microscopic features are also assessed. These include the presence and distribution of microinfarcts, white matter pallor without obvious associated vascular disease, and pallor of myelin associated with microinfarcts and arterioarteriolarsclerosis, a condition sometimes called "Binswanger's disease."

Because changes associated with Parkinson's disease are frequently present in patients with AD,[20-22] the substantia nigra is evaluated for Lewy bodies, neuronal loss, gliosis, extraneuronal neuromelanin, and neurofibrillary tangles. The frequency of Lewy bodies is scored on a four-tiered system assessing the number of neurons containing one or more Lewy bodies in a single section through the substantia nigra. The presence of Lewy bodies is also determined in other regions such as brainstem and cortex. Although there is no uniformly accepted neuropathologic definition of Parkinson's disease, working definitions were established using the criteria listed in table 3.

Ranking of disorders contributing to dementia. Finally, the pathologist is asked to list all neuropathologic diagnoses and to rank all those considered to have contributed to the dementing process.

Pretest of protocol. The CERAD neuropathology protocol has been pretested by neuropathologists from 15 participating centers. The 150 autopsy brains examined for this purpose were derived from patients from the following three entry groups: *Group 1* consisted of 10 subjects enrolled into CERAD clinical studies with clinical diagnoses of probable AD (nine cases) or as control subjects (one case); in this group, CERAD clinical and neuropsychological batteries had been administered to all subjects. *Group 2* included 133 non-CERAD-assessed individuals for whom the clinical diagnosis of probable AD was made after thorough evaluation by CERAD center-affiliated physicians but not necessarily by using CERAD clinical methods. (These cases were included to provide the opportunity for the neuropathologists to become familiar with the protocol and to insure case material for study during the early phases of this program. As the CERAD longitudinal samples have increased, group 2 subjects are no longer being entered in the database.) *Group 3* included seven non-CERAD-assessed control subjects, ie, individuals over 50 years of age and free of CNS disorders who were examined by experienced physicians at CERAD centers and found to have no evidence of cognitive impairment within a year of death. To avoid bias in case selection, only consecutively accessioned autopsy cases fulfilling the above criteria were accepted.

Analysis of data. All of the completed neuropathology data forms were reviewed by one of us (S.S.M.), and questions concerning the entries were resolved by communication with the appropriate neuropathologists. The data books were then forwarded to the CERAD Data Management Center for entry and analysis.

Table 3. Diagnostic criteria for Parkinson's disease

Definite	a	Presence of Lewy bodies at any site, gliosis, neuronal loss, and depigmented substantia nigra *and* clinical diagnosis of parkinsonism
(choose one)	b	Presence of significant degeneration (gliosis, depigmentation, and neuronal loss) of the substantia nigra without Lewy bodies (in the absence of other disorders clearly explaining this change, eg, encephalitis or multisystem degeneration) *and* clinical history of parkinsonism
Uncertain		Presence of neuropathologic lesions listed above *and* absence of clinical diagnosis of parkinsonism

Working definitions of Parkinson's disease are provided to assure uniformity of assessment.

The distribution and frequency of senile plaques and neurofibrillary tangles in cases fulfilling the CERAD neuropathologic criteria for definite AD were analyzed; logistic regression procedures for ordinal data[12] were employed. In addition, as an example of the type of neuropathologic analysis that can be performed using this database, the degree of amyloid deposition in meningeal and parenchymal blood vessels was compared to the proportion of senile plaques containing amyloid cores.

Results. Neuropathologists from 15 centers submitted data on autopsy brains of 142 patients clinically diagnosed as having probable AD and eight nondemented control subjects (table 4) from groups 1 to 3 as described above. In general, the neuropathologists readily accepted the data forms and found them to be straightforward and relatively easy to use. Most of the neuropathologists reported that using the form for entry of the neuropathologic information did not add substantively to the time ordinarily spent in working up these cases.

The neuropathologic findings on the patients clinically diagnosed as having probable AD are summarized in table 5. Using CERAD neuropathology diagnostic criteria, neuropathologists determined the primary dementing illness to be definite AD in 119 of the 142 cases (83.8%). Another 13 cases (9.1%) were judged to have probable or possible AD. (One case showing only neurofibrillary tangles, striatal degeneration, and no senile plaques was characterized by the referring neuropathologist as "atypical AD," but this case does not fall within CERAD neuropathologic criteria for AD.) Thus, 132 of 142 cases (or 93%) displayed AD changes listed by the neuropathologist as the primary cause of dementia. Parkinson's disease changes were encountered in 27 (23%) of the cases with definite AD. Although some degree of cerebrovascular disease was found in about one-third of the cases with definite AD, only three patients (2%) were considered to have vascular disease as the primary cause of their dementia. The neuropathologic diagnoses in these three cases

Table 4. Distribution of demented and control subjects by age and sex

	Subjects		Age (yrs)	
	No.	Percent	Mean	Range
Demented				
Men	77	54.2	73.2	49-94
Women	65	45.8	79.9	59-95
Total	142		76.3	49-95
Controls				
Men	5	62.5	65.8	56-79
Women	3	37.5	64.0	59-70
Total	8		65	56-79

were, respectively, Binswanger's disease, multiple infarcts, and chronic vasculitis. Neuropathologists interpreted the major cause of dementia in five individual cases to be, respectively, Pick's disease, lobar atrophy, progressive supranuclear palsy, cortical degeneration of unspecified type, and corticonigral degeneration. However, these cases of cortical and corticonigral degeneration also had some degree of concomitant AD pathologic change, emphasizing the complexity of interpretation and the need for reliable data when overlapping pathologies occur. No morphologic basis for the dementia was found in one case.

In the cases fulfilling CERAD neuropathologic criteria for definite AD, there were no significant differences in the frequency of senile plaques in the three neocortical regions. We also compared the degree of deposition of vascular amyloid with the proportion of amyloid-cored plaques in neocortex. In the frontal sections, the degree of amyloid deposition in the meningeal and parenchymal blood vessels correlated positively ($p < 0.002$ and $p < 0.02$, respectively) with semiquantitative estimates of the proportion of plaques containing amyloid cores. That is, brains of patients with AD who had a high proportion of amyloid core-containing plaques often showed heavy amyloid deposition in cerebral vessels, whereas those with few to no amyloid cores in plaques tended to have little to no vascular amyloid.

The control brains from the eight subjects without cognitive impairment revealed a spectrum of findings (table 6). Three of the eight control cases showed no plaques, tangles, or amyloid angiopathy. The brain of a 69-year-old control subject with carcinoma of the colon displayed sparse neocortical plaques giving an age-related plaque score of *B* (table 1), ie, histologic findings *suggest* the diagnosis of AD. The brain of a 70-year-old nondemented patient with squamous cell carcinoma displayed frequent frontal cortical plaques giving an age-related plaque score of *C*, ie, histologic findings *indicate* the diagnosis of AD. In the absence of a clinical history of dementia, both cases were classified as possible AD (type b) using CERAD neuropathology criteria summarized in table 2. One other control case had a single neurofibrillary tangle in the temporal cortex, sparse tangles in the hippocampus and entorhinal cortex, and sparse meningeal vascular amyloid. Another brain showed only sparse tangles in the hippocampus

Table 5. Summary of neuropathology diagnoses on 142 cases clinically diagnosed as probable Alzheimer's disease

Primary dementing illness*	No. of cases	Parkinson's disease (PD) changes	Cerebro-vascular disease	Concomitant diagnoses made (no. of cases)
Definite AD	119 (84%)	27 (23% of definite AD cases)	40 cases† (34% of definite AD cases)	Binswanger's‡ (1); definite PD‡ (5); uncertain PD (19‡); Lewy body disease (5‡); lacunes (12‡); infarcts—all sizes (26‡); vascular malformation (1); petechial hemorrhages (2); meningioma (2); metastatic Ca (1); Guillain-Barré (1); chronic subdural hematoma (1); old subarachnoid hemorrhage (1); meningitis (2), caudate atrophy (1); abscess (1), hippocampal sclerosis (1)
Probable AD	10 (7%)	3	0	Uncertain PD (1); Lewy body disease (1‡); definite PD (1‡)
Possible AD	3 (2%)	0	1	Lacunes and infarct (1); hydrocephalus (1‡)
"Atypical AD"§	1	0	0	
Cerebrovasc. disease	3 (2%)	0	3	
Infarcts and lacunes (1)				Normal pressure hydrocephalus‡
Chronic vasculitis (1)				
Binswanger's dis. (1)				Possible AD‡
Cortical degeneration, unknown etiology	1	0	0	Probable AD
Corticonigral degeneration	1	0	1	Definite AD‡; infarct
Progressive supranuclear palsy	1	1	0	Uncertain PD‡
Pick's disease	1	0	0	
Lobar atrophy	1	0	0	Possible AD
Normal brain	1	0	0	N/A
Total cases	142	31	45	

* As rated by the neuropathologist (see table 2 for neuropathology definitions).
† Includes all lacunes, infarcts, microinfarcts, and Binswanger's disease; does not include amyloid angiopathy or petechial hemorrhages.
‡ Judged by the neuropathologist to have also contributed to dementia in at least some of the cases.
§ Just tangles (no plaques) and striatal degeneration.

and entorhinal cortex without plaques or amyloid angiopathy. Amyloid angiopathy alone was found in an additional control case without plaques or tangles.

Discussion. *The need for standardization of the neuropathology assessment in AD.* Inconsistencies in the neuropathologic assessment of AD have long been recognized. No morphologic or other gold standard for diagnosis of AD exists at this time, and the need for standardized diagnostic criteria has become apparent. In an effort to meet this need, a panel of neuropathologists in 1985 recommended using quantitative criteria based upon absolute age-related neocortical plaque counts.[13] Four years later, however, a survey of 104 neuropathologists in the United States and Canada showed that only 21% of the respondents actually applied these criteria to their cases.[14] Tierney et al[15] stated in a clinicopathologic study of 57 cases evaluating the NINCDS-ADRDA criteria for the clinical diagnosis of probable AD that, "in spite of consistency in the application of clinical criteria, the lack of agreement caused by differing neuropathologic criteria for Alzheimer's disease limits our ability to compare research protocols that use different neuropathologic criteria." Although it is not the intention of CERAD to impose any absolute diagnostic criteria, our protocol addresses this problem

Table 6. Summary of clinicopathologic features on eight control cases

Clinical diagnosis	Age	Sex	Race	Plaques	Tangles	Vascular amyloid
Carcinoma of the colon; enrolled in AD study as control patient; lucid correspondence from patient 7 mos before death	69	Male	White	Sparse in neocortex, hippocampus, amygdala; moderate in entorhinal cortex	Sparse in hippocampus and entorhinal cortex; none in amygdala	None
Squamous cell carcinoma of esophagus; alert and oriented 1 mo before death	56	Male	Black	None in neocortex, hippocampus, entorhinal cortex; amygdala not examined	None in neocortex, hippocampus, entorhinal cortex; amygdala not examined	None
Squamous cell carcinoma; normal mental status described immediately before death	70	Female	Black	Frequent* in frontal cortex; moderate in temporal and parietal cortex; sparse in entorhinal cortex; none in hippocampus	None	None
Rheumatoid arthritis; chronic emphysema; cognitively normal on testing 3 mos before death	64	Male	White	None	None	Sparse to moderate parenchymal and meningeal deposition hippocampus, amygdala, and neocortex
Acute myelomonocytic leukemia; alert and oriented when seen by neurologist 2 mos before death	61	Male	White	None	None	None
Metastatic large cell carcinoma of lung	59	Female	White	None	None	None
Ameloblastoma of mandible metastatic to liver and lungs	63	Female	White	None	Sparse in hippocampus and entorhinal cortex	None
Carcinoma of pancreas and metastasis to brain	79	Male	White	None	Sparse in hippocampus, entorhinal cortex, and temporal cortex	Sparse meningeal amyloid

* Most small primitive plaques.

by offering a uniform method for the neuropathologic assessment of AD.

There is no doubt that variation exists among neuropathology laboratories regarding both histologic techniques and interpretation used to assess AD. In surveying CERAD neuropathologists, we learned that 15 centers used seven different staining techniques on brain tissue sections ranging from 6 to 15 micrometers in thickness. Even when common stains are used (such as the Bielschowsky method), modifications abound. In many laboratories, specific staining techniques are a virtual tradition; technicians and pathologists alike feel comfortable in their use and interpretation. Although brain tissue riddled with neocortical neuritic plaques probably would present no diagnostic problem to any of the participating neuropathologists, we believe that differences in technique and interpretation of the morphologic findings may yield disparate inter-center data and conclusions.

This notion was strengthened by the survey by Wisniewski et al.[14] They tabulated the diverse methodologic and interpretive approaches used by 104 neuropathologists in evaluating cases clinically diagnosed as AD. Methods listed by respondents included some Bielschowsky method (51%) and thioflavine S staining (13%), although 42% used other silver or amyloid preparations. This methodologic inconsistency was paralleled by variation in methods of evaluation of observations. Most of the neuropathologists did not use a strictly quantitative assessment to make the diagnosis of AD. Moreover, there was much variation in the extent to which they incorporated the clinical information in their diagnostic evaluation.

In an earlier position paper, Ball[16] made a similar point regarding the variations in methodology used in the assessment of AD. He stated "optimal comparison of quantitative data in the neuropathological diagnosis would involve the standardization of various fixation, processing, and staining techniques used by participating laboratories. Considerable debate persists about the

best type of histological method to show the various Alzheimer lesions. This goal could be achieved, but only with a large amount of planned cooperation." Indeed, the literature is replete with observations based upon varying methodologies. The CERAD neuropathologists are concerned about intercenter variation in staining technique and interpretation and are currently completing a standardization study addressing this important issue.[11]

Potential uses of the CERAD neuropathology protocol. The CERAD neuropathology protocol was designed to create a database that has many potential uses, including the refinement of diagnostic criteria, the assessment of overlapping and coexistent pathology, and the understanding of early changes in AD.

Refinement of diagnostic criteria. The inclusion of a wide range of neuropathologic data correlated with clinical information provided by the CERAD clinical batteries may allow refinement of diagnostic criteria. For example, as suggested by Tomlinson,[17] other more reliable diagnostic indicators may be found, eg, entorhinal or brainstem neurofibrillary tangles. The flexibility of the CERAD neuropathology protocol will enable us to take advantage of new or improved histopathologic, immunocytochemical, or other techniques.

Heterogeneity. A large-scale longitudinal study like CERAD should be well equipped to deal with the issue of clinical and neuropathologic heterogeneity in AD. Clinical features such as extrapyramidal signs or early versus late language impairment may be paralleled by distinctive patterns of neuropathology. Mayeux et al[18] and Chui et al[19] described a substantive subset of AD patients with extrapyramidal signs in the absence of neuroleptics along with severe intellectual and functional decline. These workers stressed the need for pathologic correlation but, thus far, such correlative studies have been limited. Some investigators have suggested that extrapyramidal signs in AD, especially rigidity, are related to coexistent Parkinson's disease pathology[20-22]; Morris et al,[23] however, have found heterogeneous pathologic correlates of clinical parkinsonism in AD.

Data on extrapyramidal signs are recorded in the CERAD clinical assessment forms and will be correlated with information in the neuropathology protocol on the substantia nigra as well as the presence and distribution of Lewy bodies. Such correlations will permit us to look at clinical and neuropathologic correlates of Parkinson's disease changes, either coexisting with AD pathology or occurring alone. The CERAD neuropathology protocol also may answer questions about so-called "diffuse Lewy body disease" often seen in the clinical and neuropathologic setting of AD[24] and may establish whether or not such cases represent a clinically distinctive variant of AD.[25]

The CERAD protocol will provide valuable data on the controversial role of cerebrovascular disease in dementia patients. Estimates of the percentage of dementia patients with significant cerebrovascular disease vary[26,27] and have been estimated as being as high as one-third.[28] Some workers maintain that patients diagnosed as having multi-infarct dementia or a combination of vascular disease and AD show predominantly AD or mixed neuropathology[27,29]; postmortem studies revealed a diagnostic accuracy of the clinical diagnosis of vascular dementia as 85% in a Finnish study of demented patients by Erkinjuntti and coworkers.[30] However, in a neuropathologic study of patients clinically diagnosed as having AD, Joachim et al[31] found that only two of 150 cases showed a purely vascular basis of dementia.

Although the CERAD database will include only those patients clinically diagnosed as having probable or possible AD,[3] it will provide information on the extent to which vascular disease coexists with or mimics AD. The neuropathology data form was designed to include information on the size, location, and nature of the gross and microscopic vascular lesions to help resolve some of these questions. This will be particularly valuable when combined with data obtained using the CERAD neuroimaging protocol currently in development.

Early changes of AD. Correlation of CERAD clinical and neuropsychological findings with the distribution of AD changes in patients with mild dementia or short duration of symptoms and in nondemented control subjects may reveal hierarchical patterns in the nature or in the distribution of early neuropathologic changes. In an attempt to provide clues to these early changes, neuropathologic findings in autopsies of relatively young patients with Down's syndrome have been described.[32-34]

Most of the patients entered into the CERAD clinical protocol, however, will be in the more advanced stages of AD by the time of death. Berg et al[35] emphasized the rapidity with which most AD patients move from the stage of mild to moderate or severe dementia. It is likely, therefore, that we may be largely dependent upon follow-up of CERAD control subjects as a source of early dementia cases.

Note: Requests for information about CERAD and its copyrighted assessment batteries should be directed to Albert Heyman, MD, Duke University Medical Center, Box 3203, Durham, NC 27710.

Acknowledgments

In addition to the authors, the following individuals provided helpful input into the development of the neuropathology protocol and contributed data from case material to the CERAD data bank: Melvyn J. Ball, MD, University of Western Ontario, London, ON, Canada; Linda M. Bierer, MD, Bronx VA Medical Center, New York, NY; Diana Claasen, MD, University of Pittsburgh, Pittsburgh, PA; Lawrence Hansen, MD, University of California at San Diego, La Jolla, CA; Michael Hart, MD, University of Iowa, Iowa City, IA; John Hedreen, MD, Johns Hopkins Hospital, Baltimore, MD; Victor Henderson, MD, University of Southern California, Los Angeles, CA; Bradley T. Hyman, MD, PhD, Massachusetts General Hospital, Harvard University, Boston, MA; Catharine Joachim, MD, Brigham and Women's Hospital, Harvard University, Boston, MA; William Markesbery, MD, University of Kentucky, Lexington, KY; A. Julio Martinez, MD, University of Pittsburgh, Pittsburgh, PA; Ann McKee, MD, Massachusetts General Hospital, Harvard University, Boston, MA; Carol Miller, MD, University of Southern California, Los Angeles, CA; John Moossy, MD, University of Pittsburgh, Pittsburgh, PA; David Nochlin, MD, University of Washington, Seattle, WA;

Daniel Perl, MD, Mt. Sinai Medical Center, New York, NY; Carol Petito, MD, Cornell Medical Center, New York, NY; Gutti R. Rao, MD, University of Pittsburgh, Pittsburgh, PA; Robert L. Schelper, MD, University of Iowa, Iowa City, IA; Ursula Slager, MD, University of Southern California, Los Angeles, CA; and Robert D. Terry, MD, University of California at San Diego, La Jolla, CA.

The authors thank John Morris, MD, and Gerda Fillenbaum, PhD, for thoughtful review of the manuscript and Sylvia Wrobel, PhD, and Florence Nash for editorial assistance. Duane Beekly helped with data management. Jan Fowler and Mary Strickland provided fine administrative and secretarial support and Adina Alazraki assisted with the computer-generated graphics.

References

1. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology 1989;39:1159-1165.
2. Mirra SS, Vogel FS, Hughes JP, Brownlee LM, Heyman A. The CERAD protocol for the neuropathologic assessment of Alzheimer's disease and related disorders [Abstract]. Neurology 1988;38(suppl 1):413.
3. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.
4. Dickson DW, Farlo J, Davies P, Crystal H, Fuld P, Yen S-HC. Alzheimer's disease: a double-labeling immunohistochemical study of senile plaques. Am J Pathol 1988;132:86-101.
5. Wisniewski HM, Bancher C, Barcikowska M, Wen GY, Currie J. Spectrum of morphological appearance of amyloid deposits in Alzheimer's disease. Acta Neuropathol (Berl) 1989;78:337-347.
6. Ishii T, Kamentani F, Haga S, Sato M. The immunohistochemical demonstration of subsequences of the precursor of the amyloid A4 protein in senile plaques in Alzheimer's disease. Neuropathol Appl Neurobiol 1989;15:135-147.
7. Wisniewski HM, Wen GY, Kim KS. Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol (Berl) 1989;78:22-27.
8. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Ihara Y. A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by beta protein immunostaining. Acta Neuropathol (Berl) 1988;76:541-549.
9. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Harigaya H. Diffuse type of senile plaques in the brains of Alzheimer-type dementia. Acta Neuropathol (Berl) 1988;77:113-119.
10. Joachim CL, Morris JH, Selkoe DJ. Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am J Pathol 1989;135:309-319.
11. Mirra SS, McKeel DW, Crain BJ, Hughes JP, van Belle G, Heyman A, and participating neuropathologists. Quality assurance in the neuropathology assessment of Alzheimer's disease: a multicenter study of the Consortium to Establish a Registry for Alzheimer's disease [Abstract]. J Neuropathol Exp Neurol 1990;49:336.
12. McCullagh P, Nelder JA. Generalized linear models. London: Chapman and Hall, 1984.
13. Khachaturian ZS. Diagnosis of Alzheimer's disease. Arch Neurol 1985;42:1097-1105.
14. Wisniewski HM, Rabe A, Zigman W, Silverman W. Editorial: neuropathological diagnosis of Alzheimer disease. J Neuropathol Exp Neurol 1989;48:606-609.
15. Tierney MC, Fisher RH, Lewis AJ, et al. The NINCDS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease: a clinicopathologic study of 57 cases. Neurology 1988;38:359-364.
16. Ball MJ. The pathological diagnosis of Alzheimer's disease: present neuropathological standards and future challenges. In: Hovaguimian T, ed. Alzheimer's disease: international prospectives on diagnosis. Bern, Switzerland: Hans Huber Verlag, 1987.
17. Tomlinson BE. The neuropathology of Alzheimer's disease—issues in need of resolution (second Dorothy S. Russell memorial lecture). Neuropathol Appl Neurobiol 1989;15:491-512.
18. Mayeux R, Stern Y, Spanton S. Hetrogeneity in dementia of the Alzheimer type: evidence of subgroups. Neurology 1985;35:453-461.
19. Chui HC, Teng EL, Henderson VW, Moy AC. Clinical subtypes of dementia of the Alzheimer type. Neurology 1985;35:1544-1550.
20. Leverenz J; Sumi SM. Parkinson's disease in patients with Alzheimer's disease. Arch Neurol 1986;43:662-664.
21. Ditter SM, Mirra SS. Neuropathologic and clinical features of Parkinson's disease in Alzheimer's disease patients. Neurology 1987;37:754-760.
22. Ditter SM, Brownlee LM, Mirra SS. Clinical-pathologic correlates of Parkinson's disease features in Alzheimer's disease [Abstract]. Neurology 1988;38:(suppl 1):231.
23. Morris JC, Drazner M, Fulling K, Grant EA, Goldring J. Clinical and pathological aspects of parkinsonism in Alzheimer's disease: a role for extranigral factors? Arch Neurol 1989;46:651-657.
24. Hansèn LA, Masliah E, Terry RD, Mirra SS. A neuropathologic subset of Alzheimer's disease with concomitant Lewy body disease and spongiform change. Acta Neuropathol (Berl) 1989;78:194-201.
25. Hansen LA, Salmon D, Galasko D, et al. The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. Neurology 1990;40:1-8.
26. Alafuzoff I, Iqbal K, Friden H, Adolfsson R, Winblad B. Histopathological criteria for progressive dementia disorders: clinical-pathological correlation and classification by multivariate data analysis. Acta Neuropathol (Berl) 1987;74:209-225.
27. Wade JPH, Mirsen TR, Hachinski VC, Fisman M, Lau C, Merskey H. The clinical diagnosis of Alzheimer's disease. Arch Neurol 1987;44:24-29.
28. O'Brien MD. Vascular dementia is underdiagnosed. Arch Neurol 1988;45:797-798.
29. Brust JCM. Vascular dementia is overdiagnosed. Arch Neurol 1988;45:799-801.
30. Erkinjuntti T, Haltia M, Palo J, Sulkava R, Paetau A. Accuracy of the clinical diagnosis of vascular dementia: a prospective clinical and post-mortem neuropathological study. J Neurol Neurosurg Psychiatry 1988;51:1037-1044.
31. Joachim CL, Morris JH, Selkoe DJ. Clinically diagnosed Alzheimer's disease: autopsy results in 150 cases. Ann Neurol 1988;24:50-56.
32. Mann DMA, Esiri MM. The site of the earliest lesions of Alzheimer's disease. N Engl J Med 1988;318:89-90.
33. Motte J, Williams RS. Age-related changes in the density and morphology of plaques and neurofibrillary tangles in Down syndrome brain. Acta Neuropathol (Berl) 1989;77:535-546.
34. Mann DMA, Brown A, Prinja D, et al. An analysis of the morphology of senile plaques in Down's syndrome patients of different ages using immunocytochemical and lectin histochemical techniques. Neuropathol Appl Neurobiol 1989;15:317-329.
35. Berg L, Miller JP, Storandt M, et al. Mild senile dementia of the Alzheimer type. 2. Longitudinal assessment. Ann Neurol 1988;23:477-484.

EXHIBIT B

Alzheimer's disease

Etiologies, pathophysiology, cognitive reserve, and treatment opportunities

Jeffrey L. Cummings, MD; Harry V. Vinters, MD; Gregory M. Cole, PhD; and Zaven S. Khachaturian, PhD

Article abstract—Alzheimer's disease (AD) can be diagnosed with a considerable degree of accuracy. In some centers, clinical diagnosis predicts the autopsy diagnosis with 90% certainty in series reported from academic centers. The characteristic histopathologic changes at autopsy include neurofibrillary tangles, neuritic plaques, neuronal loss, and amyloid angiopathy. Mutations on chromosomes 21, 14, and 1 cause familial AD. Risk factors for AD include advanced age, lower intelligence, small head size, and history of head trauma; female gender may confer additional risks. Susceptibility genes do not cause the disease by themselves but, in combination with other genes or epigenetic factors, modulate the age of onset and increase the probability of developing AD. Among several putative susceptibility genes (on chromosomes 19, 12, and 6), the role of apolipoprotein E (ApoE) on chromosome 19 has been repeatedly confirmed. Protective factors include ApoE-2 genotype, history of estrogen replacement therapy in postmenopausal women, higher educational level, and history of use of nonsteroidal anti-inflammatory agents. The most proximal brain events associated with the clinical expression of dementia are progressive neuronal dysfunction and loss of neurons in specific regions of the brain. Although the cascade of antecedent events leading to the final common path of neurodegeneration must be determined in greater detail, the accumulation of stable amyloid is increasingly widely accepted as a central pathogenetic event. All mutations known to cause AD increase the production of β-amyloid peptide. This protein is derived from amyloid precursor protein and, when aggregated in a β-pleated sheet configuration, is neurotoxic and forms the core of neuritic plaques. Nerve cell loss in selected nuclei leads to neurochemical deficiencies, and the combination of neuronal loss and neurotransmitter deficits leads to the appearance of the dementia syndrome. The destructive aspects include neurochemical deficits that disrupt cell-to-cell communications, abnormal synthesis and accumulation of cytoskeletal proteins (e.g., τ), loss of synapses, pruning of dendrites, damage through oxidative metabolism, and cell death. The concepts of cognitive reserve and symptom thresholds may explain the effects of education, intelligence, and brain size on the occurrence and timing of AD symptoms. Advances in understanding the pathogenetic cascade of events that characterize AD provide a framework for early detection and therapeutic interventions, including transmitter replacement therapies, antioxidants, anti-inflammatory agents, estrogens, nerve growth factor, and drugs that prevent amyloid formation in the brain.

NEUROLOGY 1998;51(Suppl 1):S2–S17

Since the early 1980s there have been dramatic advances in understanding the pathogenesis of Alzheimer's disease (AD). Several mutations associated with familial forms of AD have been identified, epidemiologic and genetic risk factors have been described, the underlying molecular biology of the classic histopathologic hallmarks of AD is increasingly well understood, and the neurochemical abnormalities characteristic of AD are better characterized. In addition, protective factors that appear to delay the onset of symptoms have been found, suggesting that the threshold for symptom appearance can be modulated to increase or decrease the risk for AD in individual patients. As the sequential steps in the cascade of events that mediate the effect of risk factors or mutations have been delineated, treatment opportunities also have emerged. Each step in the process represents a potential therapeutic target that may prevent the occurrence, defer the onset, slow the progress, or improve the symptoms of AD. Advances in understanding AD have been so marked that they require a reconceptualization of the illness with an integration of developmental and late-life factors. This review updates the recent advances in understanding the genetic mutations, risk factors, role of amyloid, histopathologic characteristics, and neurochemical abnormalities of AD. This framework defines the agenda for therapeutic research in AD.

Definition of AD. The definition of AD may vary according to the purposes for which it is used. A variety of criteria can be applied for clinical diagnoses, therapeutic trials, epidemiologic research, or

From the Departments of Neurology, Psychiatry and Biobehavioral Sciences (Dr. Cummings), and Pathology and Laboratory Medicine (Neuropathology Division) (Dr. Vinters), University of California at Los Angeles School of Medicine, CA; the Medicine Service, Sepulveda Veterans Affairs Medical Center (Dr. Cole), Sepulveda, CA; and Khachaturian, Radebaugh, & Associates, Inc. (Dr. Khachaturian), Potomac, MD.

Address correspondence and reprint requests to Dr. Jeffrey L. Cummings, UCLA Department of Neurology, 710 Westwood Plaza, Los Angeles, CA 90095-1769.

pathologic investigations.[1] The most broadly applied criteria for the clinical definition of AD were introduced by the Work Group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).[2] These criteria classify AD into definite, probable, and possible levels of diagnostic certainty. The diagnosis of definite AD requires both the clinical features of probable AD and histopathologic confirmation by biopsy or autopsy. Probable AD requires the presence of dementia established by clinical examination, documented by standardized mental status assessment, and confirmed by neuropsychological tests. These must demonstrate deficits in two or more areas of cognition, with progressive worsening of memory and other cognitive functions in the absence of delirium. The onset must be between the ages of 40 and 90 years, and there must be no other systemic or brain diseases that could account for the progressive deficits in memory and cognition. Features supportive of the diagnosis of probable AD, but not required, include progressive deficits in specific cognitive functions such as language, praxis, and perceptual recognition; impaired activities of daily living; a family history of a similar disorder, particularly if confirmed pathologically; and normal or nonspecific results on routine tests such as spinal fluid examination, electroencephalography, and computerized tomography. Features consistent with the diagnosis of probable AD include psychiatric and behavioral abnormalities, weight loss and, in advanced stages of the disease, increased muscle tone, myoclonus, gait abnormalities, and seizures.

The diagnosis of possible AD is made when the typical clinical syndrome is present but there are variations in the onset, presentation, or clinical course; when a second systemic or brain disease is present but is not considered to be the cause of the dementia; and when a single, gradually progressive cognitive deficit is identified in the absence of another identifiable cause. Features noted to make AD unlikely include a sudden apoplectic onset, focal neurologic abnormalities, or seizures or gait disturbances at the onset or early in the course of the illness. Investigation of these criteria has shown them to have moderate to good reliability and validity.[3,4]

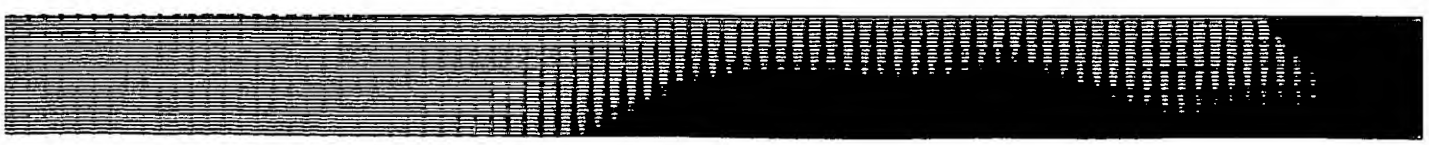
Several features of this clinical definition warrant elaboration in the context of understanding the associated etiologies and pathophysiologies of AD. First, probable AD is a clinical syndrome that has several etiologies. If each mutation is viewed as a distinctive cause of AD, then the disease has many etiologies that produce a common clinical phenotype. Second, the core syndrome is defined on the basis of an inclusionary rather than an exclusionary approach. Third, the NINCDS-ADRDA criteria define a core syndrome requiring progressive deficits in memory and at least one other cognitive domain. However, these criteria allow substantial clinical heterogeneity in terms of the age of onset, profile of cognitive abnormalities, speed of progression, and associated behavioral disturbances. Fourth, the criteria do not identify a biologic marker of the illness, and no definitive biologic diagnostic test has evolved since these criteria were published. Fifth, the onset of the disease is insidious and the course gradually progressive. This suggests that mild symptoms may be present for substantial periods of time before the family becomes aware of or sufficiently alarmed about behavioral and neuropsychological abnormalities to seek clinical consultation. Furthermore, the disease may be present for many years before any symptoms appear.

Neuroimaging studies of patients at genetic risk for AD suggest that brain changes precede the appearance of the disease by several years and are present when patients show no evidence of a dementia syndrome.[5] Studies in other neurodegenerative diseases, such as Parkinson's disease, indicate that as many as 80% of the neurons must be dysfunctional before a symptomatic threshold is crossed and clinically detectable abnormalities appear.[6]

Histopathology of AD. The histopathology of Alzheimer's disease includes neuritic plaques, neurofibrillary tangles, loss of synapses and neurons, granulovacuolar degeneration, AMY plaques, and amyloid angiopathy.

Three types of amyloid-related plaques are recognized in the brain of the AD patient.[7,8] Diffuse plaques contain no amyloid core but contain poorly circumscribed stable amyloid (Aβ)-immunoreactive protein[9] and are presumed to represent the earliest stage of plaque formation. These are found in areas of the brain that are not symptomatic in the course of AD, such as the cerebellum, and throughout the cerebral hemispheres.

The classical neuritic plaque (figure 1) is a spherical structure 50 to 200 μm in diameter, consisting of a central A-immunoreactive amyloid core surrounded by dystrophic neurites. The neurites often contain paired helical filaments, normal glial processes, and abnormal organelles. Reactive astrocytes and microglia are also found within the plaque and at the plaque periphery.[10,11] Organelles contained in the dystrophic neurites include lysosomes, mitochondria, and synaptic vesicles. In addition to amyloid, neuritic plaques include τ protein, α_1-antichymotrypsin, apolipoprotein E (ApoE), and glycosaminoglycans, among other components. Neurotransmitters and transmitter-related enzymes are also present.[12] Diffuse plaques contain amyloid but are smaller and do not contain neurites or evidence of adjacent neuronal injury. The third type of plaque, classically described as the "burnt out" plaque, consists of an isolated dense amyloid core. Until recently, criteria for the histopathologic diagnosis of AD were based on the number of neuritic plaques present in the cortex in relation to age-related standards.[13] These criteria have been revised by a work group jointly sponsored by the National Institute on



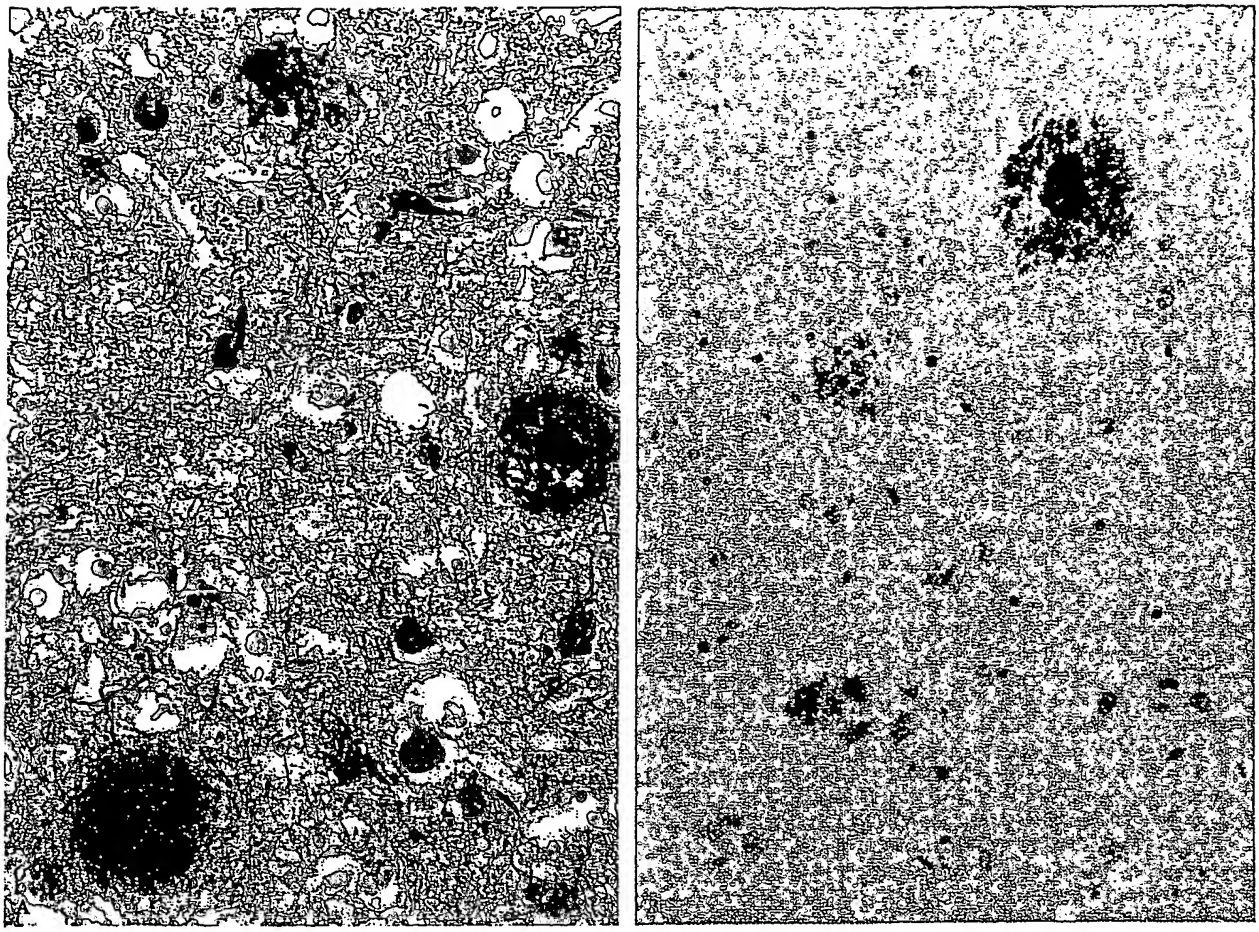


Figure 1. Neuritic plaques (NP). (A) Neuritic plaques in a section of temporal cortex from an AD patient stained with the Bielschowsky technique. The plaque at right shows both neuritic and amyloid "core" components, whereas the plaque at bottom shows only neurites, which may represent absence of an amyloid core or a tangential cut through a core-bearing plaque. Neurofibrillary tangles also are seen within the field. (B) Section of AD cortex immunostained with an antibody to Aβ 42. Note prominent immunoreactivity of both the core and the neuritic "halo" of the plaque at the top of the micrograph. The other two regions of immunoreactivity (middle and bottom) represent either diffuse plaques or tangential sections through larger core-bearing plaques (×105).

Aging and the Reagan Institute of the Alzheimer's Association. The new criteria emphasize the presence of both neuritic plaques and neurofibrillary tangles in the neurocortex.[14]

Evidence of inflammation is demonstrable within or immediately adjacent to the neuritic plaque. Acute phase reactants, such as α_1-antichymotrypsin and α_2-macroglobulin, are present in neuritic plaques, and activated microglia immunopositive for interleukin-1 and interleukin-6 also are detectable (figure 2). The complement system is activated in neuritic plaques.[15,16] Inflammatory activity within the plaque may influence amyloid metabolism and increase neuronal death.

Neurofibrillary tangles (figure 3) represent another characteristic histopathologic change observed in AD. These tangles consist of paired helical filaments that occupy the cell body and may extend into the dendrites but do not occur in the axon. Paired helical filaments consist of protofilaments arranged to form a tubule and containing abnormally phosphorylated τ-protein. Tangles exhibit immunoreactivity against antigen A-68, casein kinase-2, protease nexin-1, fibroblast growth factor, microtubule-associated protein-5 (MAP-5), ubiquitin, and β-amyloid (Aβ).[12,17] Extraneuronal "ghost" tangles may be seen in the entorhinal cortex and represent the insoluble residue of neurons that have died.[12] Large pyramidal cells are the neurons most likely to develop neurofibrillary tangles, particularly those with long, ipsilateral cortical–cortical connections.[17] The evolution of the distribution of neurofibrillary tangles within the cortex is systematic, beginning in the transentorhinal cortex, progressing to limbic cortical regions, and finally reaching neocortical areas.[17] This pattern correlates with the early occurrence of memory abnormalities in AD, followed by aphasia and apraxia, reflecting involvement of heterocortical

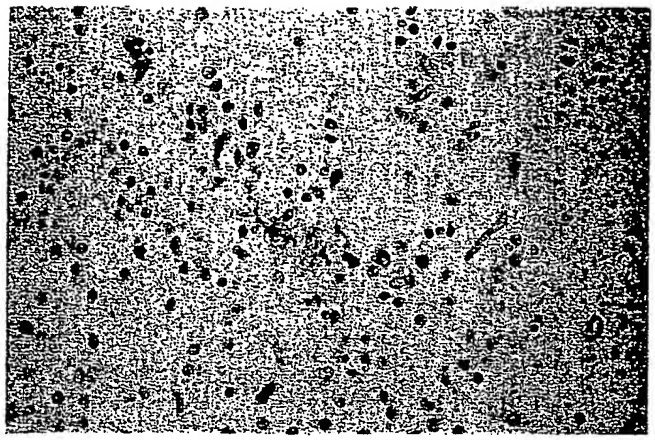


Figure 2. Section of AD brain immunostained with an antibody (HAM-56) that labels microglia, macrophages, and vascular endothelium. Note (center) ramified microglial cell and other immunoreactive cells, possibly representing macrophages. Adjacent capillary endothelium is also prominently immunoreactive (×104).

neurons that have the longest intrahemispheric projections. Early and increasingly severe involvement of limbic cortex is associated with a broad range of neuropsychiatric symptoms.[19] Neurofibrillary tangles lead to the death of nerve cells in which they occur, but nerve cell death in AD also occurs in regions with few tangles. Neurofibrillary tangles occur in non-AD CNS diseases, including progressive su-

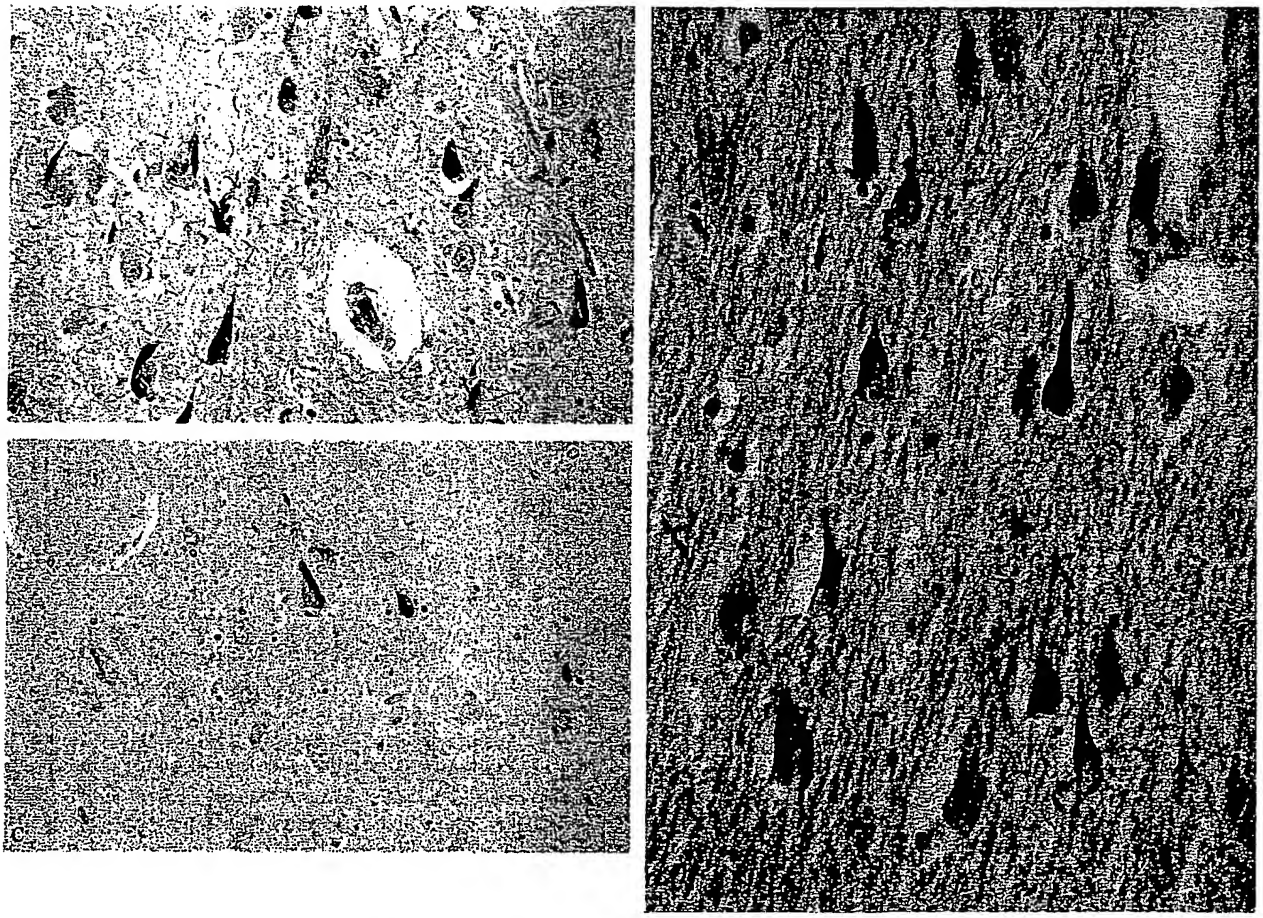


Figure 3. Neurofibrillary tangles (NFT). (A) Bielschowsky-stained section of hippocampus (AD brain) in which there are prominent flame-shaped neurofibrillary tangles in viable neurons, as judged by the presence of nuclei in affected cells. Neurons showing granulovacuolar degeneration (arrows) also are seen. (B) A field (Bielschowsky-stained section) with neurofibrillary tangles only. (C) Section of AD brain immunostained with anti-ubiquitin, which highlights prominently immunoreactive neurofibrillary tangles. Punctate immunoreactivity throughout the neuropil represents neurites or neuropil threads (×104).

pranuclear palsy, postencephalitic Parkinson's disease, dementia pugilistica, and subacute sclerosing panencephalitis,[16] and have been observed in neurons of dysplastic cortex resected from children with intractable early-onset epilepsy.[20] Therefore, neurofibrillary tangles are less specific to AD than neuritic plaques, which are almost unique to AD and to so-called normal aging. Correlations between either neuritic plaque or neurofibrillary tangle density and dementia severity are usually limited. In normal elderly, dementia severity correlates more with the presence of neurofibrillary tangles than with the abundance of neuritic plaques.[21]

In addition to amyloid-containing plaques and tangle-bearing neurons, the brains of AD patients have abundant AMY plaques. Monoclonal antibodies reveal a protein that is coexistent with but distinct from hyperphosphorylated τ-protein. AMY plaques contain no amyloid, although they are found only in brains that also have Aβ and occur in the same brain regions as amyloid plaques.[22]

Nerve cell loss, particularly affecting the larger neurons of the superficial cortex, is a consistent feature of AD.[7,8,12,16] Neuronal loss is more severe in younger than in older patients and is associated with an increased number of astrocytes.

Synaptic alterations also are characteristic of AD. Presynaptic terminal density is decreased by an average of 45% at the time of autopsy.[16] Whereas synapses are not diminished in the area of diffuse plaques, they are greatly reduced in the region of neuritic plaques. Correlations between loss of synapses and cognitive alterations in AD exceed those reported for plaques and tangles.[23]

In AD, amyloid is deposited in cerebral blood vessels and in diffuse and neuritic plaques. It involves the leptomeningeal and superficial cortical vessels.[24,25] The location of the cerebral amyloid angiopathy (CAA) is not associated with a local concentration of neuritic plaques.[26,27] However, CAA-affected microvessels may occasionally be surrounded by neuritic processes (figure 4). The presence of severe Aβ-immunoreactive CAA with relatively inconspicuous (or absent) neuritic plaques in an autosomal dominant Dutch disease, clinical features of which include cerebral hemorrhage and dementia, raises the issue of whether CAA, apart from tangles and senile plaques, can produce a dementia syndrome.[28,29] The nosologic interface between sporadic (including stroke-associated) CAA and AD is controversial.[30]

Granulovacuolar degeneration is another histologic abnormality routinely identified in AD but rare in normal aging. The involved cells occupy the pyramidal cell layer of the hippocampus. Vacuoles are present in the cytoplasm of the pyramidal cells. Each vacuole contains a single dense granule, and several vacuoles may occur within the same cell body. The granules react with anti-τ-related Alz-50 and anti-neurofilament antibodies.[16,17,31] Whereas the other lesions are neocortical and hippocampal, and sometimes involve the deep central gray structures, granuovacuolar degeneration is almost always confined to the hippocampus.

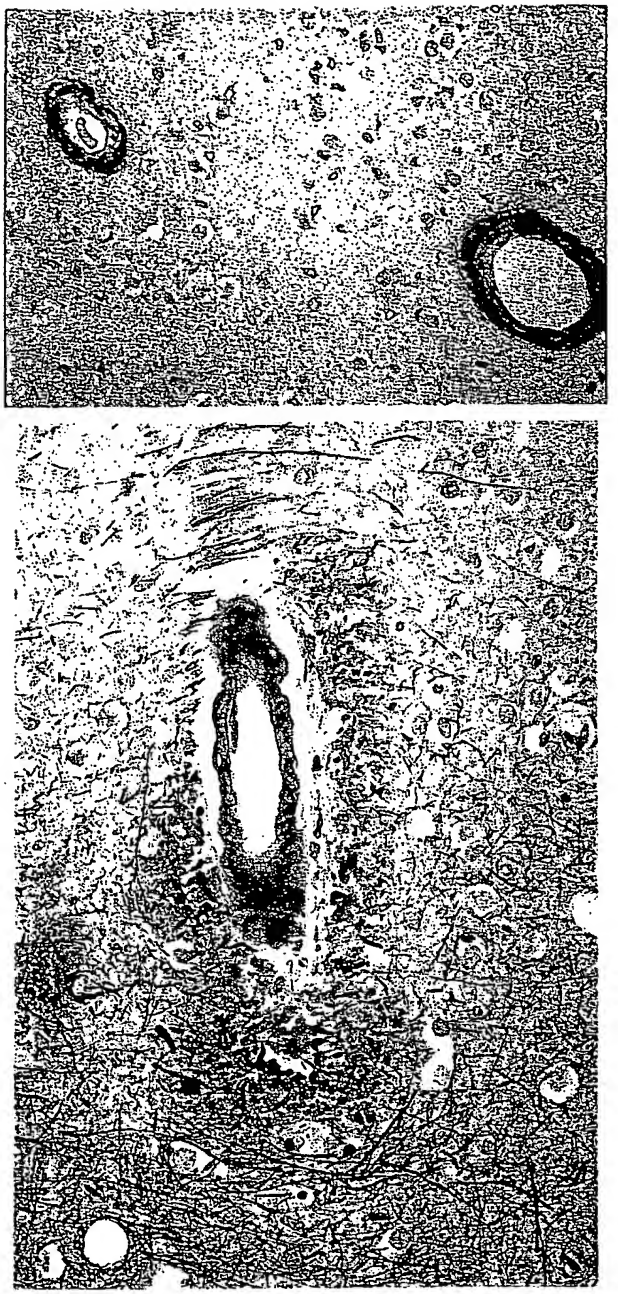


Figure 4. Congophilic amyloid angiopathy. (A) Histologic section immunostained with an antibody to a portion of β/A4. Note immunoreactivity confined to arteriole walls. (B) A Bielschowsky-stained section with an arteriole thickened by amyloid, resulting in effacement of the normal media. The vessel is surrounded by neurites similar to those seen at the periphery of neuritic plaques (×104).

Neuropil threads are processes that contain paired helical filaments and that occur primarily in

Table 1 Cortical choline acetyltransferase levels in Alzheimer's disease patients[33,34]

Cortical region	Percent reduction in choline acetyltransferase
Midfrontal cortex	58–83
Orbitofrontal cortex	80
Superior temporal cortex	61–90
Inferior parietal cortex	70–90
Cingulate gyrus	68
Hippocampus	90
Amygdala	87
Substantia nigra	78
Caudate nucleus	66
Thalamus	Normal
Midbrain	Normal
Pons	Normal
Cerebellum	Normal
Precentral gyrus	Normal
Postcentral gyrus	Normal
Occipital cortex	Normal

the dendrites of tangle-bearing pyramidal cells. They react with anti-τ and anti-ubiquitin antibodies.[16,17,32]

Neurotransmitter disturbances in acetylcholine AD. Acetylcholine (ACh) is synthesized by choline acetyltransferase (CAT), and a major source of CAT is the nucleus basalis of Meynert in the basal forebrain. This region is affected early in the course of AD, leading to a marked and consistent deficit in CAT and of ACh synthesis. Table 1 shows that CAT levels are reduced from 58 to 90%[33,34] and that reductions, particularly in the temporal lobes, correlate with the severity of the dementia syndrome.[35] Acetylcholinesterase, the enzyme responsible for the degradation of ACh, is also reduced in AD.

Muscarinic and nicotinic cholinergic receptors have been identified in the brain. Five types of muscarinic receptors are known.[36] The M1 receptor is found in hippocampus and in upper and lower levels of the cerebral cortex, whereas the M2 receptor is found in the brainstem and nucleus basalis. M1 receptors are relatively preserved in AD, whereas M2 and nicotinic receptors are markedly decreased.[37] The M1 receptors, although largely preserved in number, may not be completely functional. They appear to be partially decoupled from G-protein second-messenger systems.[37]

Other neurotransmitters and modulators. Noncholinergic transmitters and neuromodulators are also affected in AD. Serotonin is reduced by 50 to 70%, GABA is reduced by 50%, somatostatin is reduced by 40 to 60%, and norepinephrine is reduced by 30 to 70%.[38,39] Receptors for serotonin, glutamate, and somatostatin are also decreased in AD.[40]

Table 2 Mutations and vulnerability genes associated with Alzheimer's disease

Genotype	Cellular effect
Mutations	
Down syndrome (trisomy 21)	Increased APP production with enhanced generation of Aβ
APP mutations (various)	Altered APP processing resulting in increased production of Aβ
Chromosome 14 (PS1 mutation)	Increased Aβ production
Chromosome 1 (PS2 mutation)	Increased Aβ production
Genetic risk factors	
Chromosome 19 (ApoE-4)	Increased Aβ aggregation
Chromosome 12 (low-density lipoprotein receptor-related protein)	Lipoprotein receptor mediating the molecular effects of ApoE-4
Chromosome 6 (HLA-A2)	HLA histocompatibility allele regulating the inflammatory response
Chromosome 17	Bleomycin hydrolase; implicated in APP processing

APP = amyloid precursor protein; Aβ = amyloid β-protein; PS = presenilin; ApoE = apolipoprotein-E; HLA = human leukocyte antigen.

Mutations causing AD. Mutations involving chromosomes 21, 14, and 1 cause AD.[41,42] The mutations are inherited in an autosomal dominant mode of transmission and, when present, usually cause familial early-onset AD, although there is an unusually wide range in age of onset of disease in individuals with the chromosome 1 mutation.[43] In families carrying the mutations that cause AD, there is variability in age of onset and in phenotypic heterogeneity. These differences among family members carrying identical mutations suggest that environmental factors modify both the timing and the symptom profile of disease presentation.[44] Table 2 summarizes the mutations associated with AD and their effects on cellular metabolism.

Down syndrome. Down syndrome was the first genetic syndrome to be linked to AD. Down syndrome patients over the age of 30 almost universally have AD-type pathology in the brain, and the prevalence of dementia in Down syndrome also increases with age.[45] Patients with Down syndrome have three copies of chromosome 21, which bears the gene for the amyloid precursor protein (APP), and have increased production of Aβ protein, the toxic protein fragment derived from APP.[46,47]

Chromosome 21 mutations. Several mutations of the APP gene on chromosome 21 have been described in patients with early-onset familial AD. These mutations occur near the beginning and end of the Aβ peptide (i.e., at cleavage sites in the precursor pro-

tein). They affect APP processing in different ways, but all increase the production of Aβ,[47] particularly the form with 42 amino acids, which has the greatest association with neurotoxicity.[42,47,48]

Chromosome 14 (presenilin 1) mutations. Several mutations of the presenilin gene on chromosome 14 have been described, and these appear to account for a majority of cases of familial early-onset AD. These mutations also increase the production of Aβ 42.[47,48]

Chromosome 1 (presenilin 2) mutations. A small number of families who originated in Germany and migrated to the Volga River area of Russia before coming to the United States are at risk for familial AD. These individuals have mutations on chromosome 1 (presenilin 2).[49] Like the presenilin 1 mutations, those in the presenilin 2 gene are associated with increased production of Aβ.[50] The two presenilin genes are very similar; both are transmembrane proteins with six to nine transmembrane domains and a high degree of amino-acid sequence homology.[47]

The effect common to all mutations that cause AD—trisomy 21, APP mutations, presenilin 1 mutations, and presenilin 2 mutations—is the overproduction of Aβ. Moreover, it is the 42-amino-acid type of Aβ, that is increased by these mutations, and this type appears to be more toxic than other forms of amyloid.

Risk factors for AD. Mutations account for only a small number of patients with AD (less than 5%). Most of the patients with mutations have early-onset familial AD, whereas most patients with AD have dementia syndromes that begin later in life and are sporadic or occur in families without a specific autosomal dominant pattern of inheritance. Several risk factors applicable to sporadic AD have been identified, including genotypes that confer vulnerability (e.g., susceptibility genes) and epigenetic factors such as age, female gender, small head size, intelligence, educational level, history of head trauma, and as yet undetermined environmental influences.

Vulnerability genes. Three genes have been described that contribute to vulnerability to AD without having an obligatory association with the disease. Individuals carrying one of these genes are at increased risk for AD, but AD occurs in individuals without these risk factors and individuals carrying the risk factors may not develop the disease. The first such risk factor to be described was the ApoE e-4 allele. The three common alleles of ApoE are e-2, e-3, and e-4. Of these, e-3 is the most common, followed by e-4 and e-2 (table 3).[51] ApoE-4 increases the risk for developing AD and is associated with an earlier age of onset.[52] ApoE-4 increases the deposition of Aβ (primarily Aβ 40), appears to facilitate the configurational change from diffuse to aggregated amyloid in a β-pleated sheet configuration,[53] and is associated with an increased frequency of neuritic plaques and more marked cholinergic deficiency. Neurofibrillary tangles are not more abundant in patients with the e-4 allele.[53,54] ApoE may play an important role in growth and maintenance of the nervous system, and the e-4 allele may confer less advantage in these functions than e-2 and e-3.[55]

Table 3 Frequency of ApoE alleles in Alzheimer's disease (AD) patients and controls living in the community[51]

Genotypes	AD Patients (n = 234) (%)	Controls (n = 304) (%)
2/2	0	0
3/2	3.4	12.5
3/3	38.5	59.9
4/2	4.3	4.9
4/3	41.0	20.7
4/4	12.8	0.7
Allele frequencies (95% confidence intervals)		
2*	0.02–0.06	0.08–0.12
3*	0.56–0.65	0.73–0.80
4*	0.31–0.40	0.11–0.16

* $p < 0.001$.

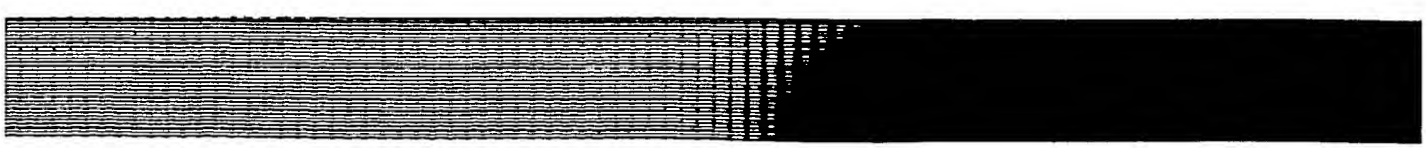
The low-density lipoprotein receptor-related protein gene (LRP), an ApoE receptor, is coded for by alleles on chromosome 12. The protein has T and C/C alleles, and the C/C allele is associated with an earlier age of onset and a significantly greater abundance of neuritic plaques. These effects were demonstrable even in the absence of an e-4 allele, suggesting that other ligands of the receptor protein may be associated with AD.[56] These observations are provocative and require confirmation before the role of the LRP gene in AD can be more fully understood.

The human leukocyte antigen (HLA)-A2 allele on chromosome 6 has been associated with an earlier age at onset of AD.[57] The HLA alleles comprise a tightly linked group of genes that form the major histocompatibility complex in humans and regulate the immune response. The immunologic aspects of AD may be mediated in part by these HLA genes, with the A2 allele conferring a risk for earlier onset of AD. Further studies of this association are warranted.

Bleomycin hydrolase, encoded on chomosome 17, resembles proteases implicated in APP processing and have been associated with AP suseptibility.[58]

Family history of AD. A family history of AD is a risk factor for developing the disease, and the risk extends beyond those family members with identified risk factors such as mutations or an ApoE-4 genotype. When followed into their late 80s or 90s, approximately 50% of first-degree relatives of AD patients will have developed the disorder. This frequency is two- to four-fold the risk for elderly individuals without a family history of AD.[59-60]

Age. Age is a powerful risk factor for AD. Both the prevalence and the incidence of AD double approximately every 5 years after the age of 60. Prevalence rises from approximately 1% in 60- to 64-year-olds to 2% in those aged 65 to 69, 4% in those aged



70 to 74, 8% in those 75 to 79, 16% in those aged 80 to 85, and approximately 35 to 40% in those over the age of 85.[61,62] Similarly, the incidence increases from approximately 2.5% for subjects aged 75 to 79 to 5% for those 80 to 85 and almost 10% for those aged 85 and older.[63] The overall prevalence of AD after the age of 65 is approximately 10%.

Among the neurobiologic changes of normal aging that may contribute to the risk for AD symptoms are loss of neurons, loss of synapses, decrease in the size of the dendritic domain of neurons, reduction in neuronal number and size in the nucleus basalis of Meynert, and decrease in cortical ACh. Neuritic plaques and rare neurofibrillary tangles (confined to the hippocampus) also may occur in the course of normal aging.[64,65]

Female gender. More women live into the risk period for AD and therefore women are more common in populations of AD patients. When adjustments are made for survival, women continue to be at slightly greater risk than men for AD.[66-68]

History of head trauma. Several studies have found that previous head injury is a risk factor for AD,[69-71] and a few failed to confirm this association.[72-74] These discrepancies may be explained by a synergistic interaction of head trauma, ApoE-4 genotype, and amyloid deposition in post-traumatic encephalopathy.[75-77] There is deposition of immunoreactive Aβ in the cortical ribbon after head trauma, and this process appears to be amplified if the patient has an ApoE-4 genotype. Synaptic relationships between neurons may also be disrupted by diffuse axonal injury sustained at the time of head injury.[78] These predisposing factors increase the likelihood of the later appearance of AD.

Head circumference and brain size. Individuals with smaller heads are at increased risk for AD. This risk remains evident after adjustment for possible confounding influences such as weight, height, ApoE genotype, gender, and education.[79-81] Smaller brain size has also been associated with an earlier onset of AD.[82] Larger brains have more nerve cells with more synaptic connections, which may confer greater protection against the symptoms of AD.[83]

Intelligence. Lower cognitive function has been associated with a higher risk for AD, independent of the effect of education.[84] Higher intelligence is associated with larger brain size and more rapid nerve conduction.[85] These factors may increase cognitive reserve (see below) and thus may prevent or defer the onset of AD, whereas lower intelligence increases the likelihood of manifesting AD.

Protective factors. The age of onset or clinical detection of AD may be delayed by several exogenous and endogenous factors. These "protective" factors may be of great importance in planning rational therapies for AD.

ApoE-2 genotype. The ApoE-4 genotype increases the risk for AD; in contrast, the ApoE-2 genotype is correlated with a decreased risk for AD.[86] Patients carrying the ApoE e-2 allele also have a later onset of disease. The mechanism remains unclear, but several possible explanations have been suggested: (a) delipidated and therefore denatured ApoE-2 does not interact with Aβ; (b) ApoE-2 may protect against the formation of intraneuronal neurofibrillary tangles[87]; (c) ApoE-2 is reported to have neuroprotective antioxidant activity[88]; and (d) lipid-bearing native ApoE-2 and ApoE-3 bind Aβ very well but ApoE-4 binds Aβ poorly.[89,90] This may allow native E2 and E3 to promote successful Aβ clearance bound to ApoE rather than self-aggregation and deposition. Because ApoE-2 binds neuronal receptors for ApoE much less efficiently than ApoE-3 and ApoE-4,[91] ApoE-2 may exert neuroprotective effects by failing to deliver Aβ to neurons.

Nonsteroidal anti-inflammatory drugs. Several studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a lower risk for AD.[92-96] In addition, NSAIDs have been associated with a slower rate of cognitive decline in patients with the disease.[97] The effect of NSAIDs may be mediated through their impact on inflammatory processes that occur in neuritic plaques (particularly those processes mediated by microglia).

The principal targets of the NSAIDs in wide use that appear to reduce AD risk in epidemiologic studies include cytooxygenases (COX) I and II. It is hypothesized that major side effects arise from COX I inhibition, whereas COX II is the target of choice for reduction of inflammation. COX II inhibition is a focus of major pharmaceutical company interest.[98] The cellular targets for anti-inflammatory treatment are the reactive glia, especially microglia, which are closely associated with neuritic plaques.[15] In the CNS, however, COX II is present not only in microglia but also in neurons. COX II is reduced in AD, presumably because of neuron loss.[99] COX II inhibition in AD may directly affect neuronal function as well as the inflammatory glia.

Antihistamine agents also may have beneficial effects that reduce the risk or delay the onset of AD.[100]

Estrogen replacement therapy. Estrogen replacement therapy (ERT) in postmenopausal women has been found to confer a decreased risk for AD.[101] The risk decreased with increasing dose of estrogen and with an earlier age of menarche. Women who receive ERT have been found to perform at a higher cognitive level than those who do not.[102] Estrogens exert a variety of actions that are potentially useful in AD, including neurotrophic functions, neuroprotective effects, and benefits on cerebral blood flow.[103] Estrogen also increases CAT activity in the basal forebrain.[104] Males have lifelong sources of brain estrogen through the intracerebral conversion of testosterone to estrogen and thus avoid late-life estrogen deficiency.[103]

Education and challenging occupations. Education has a protective effect, reducing the risk for AD. Several studies have found that higher levels of education are associated with a delay in the onset of AD.[105-109] This effect is most apparent in studies com-

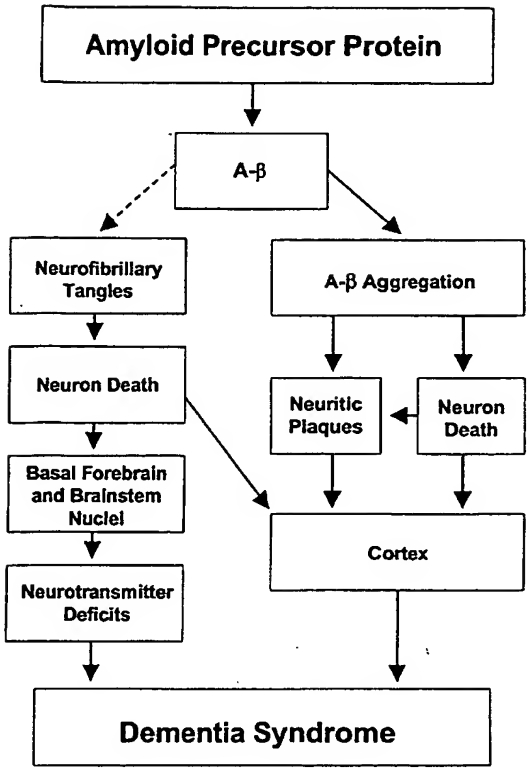


Figure 5. Hypothesized cascade of events leading from mutations or risk factors to Aβ production, nerve cell death, neurotransmitter deficiencies and the emergence of the dementia syndrome.[112,113]

paring those with little or no education to those with at least a secondary education, but some studies have shown a dose–response relationship continuing through the college education level. The effect of continuing educational activities into adult years has not been as extensively studied, but at least one investigation found a benefit of continuing participation in social and leisure activities.[110] Katzman[111] proposed that education increases synaptic density in neocortical association cortex, thus increasing cognitive reserve (discussed in detail below) and delaying the symptoms by 4 to 5 years.

The amyloid cascade hypothesis: integrating diverse risk enhancement and risk reduction factors. The amyloid cascade (figure 5)[112,113] is a sequential series of events that is hypothesized to represent the principal elements of the pathogenesis of AD. It is supported by substantial empirical evidence and permits many observations to be integrated into a unifying model. Some aspects of the model are hypothetical and remain to be studied and others are controversial; the model will evolve as additional research is conducted.

APP is a large molecule that contains the short 40–42-amino-acid Aβ fragment with its carboxyl terminus embedded in the transmembrane domain. No neurotoxicity is associated with APP per se or with non-Aβ–containing domains. A major catabolic pathway for APP begins with cleavage by an α-secretase, which cuts within the Aβ region, thus preventing production of Aβ. A normal minor pathway, which is increased by familial AD mutations, requires two cleavage events to liberate Aβ. First, a β-secretase cleaves the APP molecule at the amino terminus of Aβ. Then a γ-secretase cleaves the molecule at the carboxy terminus, releasing the Aβ fragment. This processing occurs primarily in endosomes leading to extracellular Aβ, with a minor fraction of Aβ 42 apparently produced in or near the endoplasmic reticulum and detectable as intracellular Aβ.[47,114,115] Aggregation of Aβ into β-pleated sheet conformation oligomers or fibrils correlates with the occurrence of toxicity in vitro. This fibrillar form is found in mature plaques with degenerating neurites. Although representing only about one-fifth of the total Aβ produced, the 42–amino-acid form of Aβ has two additional hydrophobic amino acids so that it aggregates into stable β-pleated sheet oligomers and fibrils much more readily than the 40–amino-acid form.[116,117] All known mutations that are apparently causally linked to AD increase the production of Aβ (specifically the 42-amino-acid form), and some of the other risk factors for AD are also associated with increased Aβ production, including normal aging and traumatic brain injury.

Amyloid β-protein is hypothesized to contribute to the clinical syndrome of dementia by adversely affecting neuron function and, ultimately, survival. The mechanisms by which this is accomplished remain unresolved, with evidence for several different hypotheses based largely on in vitro observations. Aβ increases production of H_2O_2, leading to oxidative damage and cell death.[118,119] Oxidative stress refers to the cytotoxic effects of free radicals, such as the superoxide ion, hydroxy radicals, and H_2O_2, which can be generated during normal or abnormal cellular activities that utilize molecular oxygen.[120] Oxygen radicals produce injury to neuronal membranes and to mitochondrial DNA.[121] Oxidative injury also may make neurons more vulnerable to dysfunction produced by the excitatory amino acid glutamate.[122] Free radicals may contribute to aggregation and deposition of Aβ and to binding of ApoE-4 to Aβ.[123] In addition to direct effects on neuronal oxygen radical production, Aβ-induced activation of microglia results in toxic free radical and cytokine secretion. This may further damage neurons.[124] The presence of increased lipid peroxidation and evidence for damaged mitochondria in AD cerebral cortex supports the existence of increased oxidative damage in AD.[123]

Aβ protein disturbs calcium homeostasis and increases intraneuronal calcium concentrations that may activate intracellular proteases, lipases, and other enzymes leading to cell death.[113,125,126] τ-Phosphorylation is also controlled by intracellular calcium, and Aβ may be involved through this mechanism in both cell death and promotion of

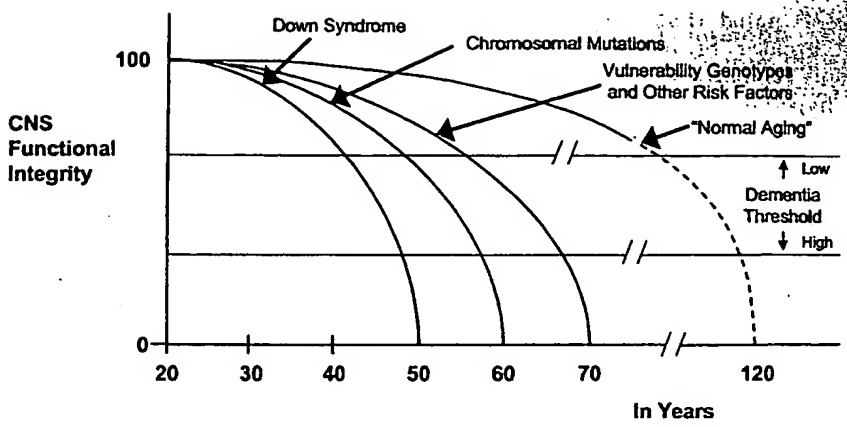


Figure 6. The interaction of the amyloid cascade and decreased brain function with cognitive reserve and symptom thresholds.

neurofibrillary tangle formation.[113] Calcium influx also may damage mitochondria, resulting in increased generation of free radicals and oxidative injury.

Unlike amyloid plaques and tangles, which appear to plateau early in the course of the disease, neuron loss continues to occur as dementia progresses in patients followed from biopsy to autopsy.[127,128] Neuronal death is not equally distributed across all cerebral hemispheric regions. The hippocampus, amygdala, and mediotemporal regions are most affected, followed by the temporo–parieto–occipital junction and then by the frontal heteromodal cortex.[129] In addition, transmitter source nuclei are affected, including the nucleus basalis of Meynert, locus ceruleus, and midbrain raphe. Disturbances in these regions lead to deficiencies in ACh, norepinephrine, and serotonin, respectively. The selective vulnerability of neurons in AD remains unexplained. Braak and Braak[18] observed that neurons with long intrahemispheric connections are the most vulnerable to injury in AD. The combined histologic and neurochemical abnormalities produce the dementia syndrome observed clinically.

Hemispheric asymmetries in the effects of neuronal and neurochemical dysfunction in AD contribute to the heterogeneity of the clinical syndrome. PET reveals that in most patients there is similar bilateral involvement of the parietal and posterior temporal regions.[129] Patients with disproportionately prominent aphasia exhibit greater left hemispheric hypometabolism, whereas those with more pronounced visuospatial deficits exhibit more profound right hemispheric abnormalities.[130,131] Patients manifesting psychosis or marked agitation have greater involvement of frontal and temporal cortex than patients without these associated behavioral disturbances.[132]

Among the first manifestations of neuronal dysfunction is the failure to maintain the most distant cellular ramifications. Therefore, loss of synaptic density would be a predictable early manifestation of neuronal alterations. Normal aging and trauma reduce synaptic connections and may exaggerate the effects of AD-related neuronal damage. Conversely, the effect of education is to increase neuronal connectivity, thus increasing the threshold between the effects of neuronal dysfunction and the appearance of symptoms. Finally, both estrogens[133] and NSAIDs[134] exert neuroprotective effects that might reduce the occurrence or ameliorate the progression of the dementia syndrome. Therefore, many of the risk factors and protective factors that have been identified in AD can be placed in the context of their effects on maintaining neuronal function and resisting the adverse effects of Aβ on neuronal survival.

Cognitive reserve and symptom thresholds. The cognitive reserve hypothesis posits that individuals manifest different thresholds for symptom occurrence with brain dysfunction. Those with a greater reserve can sustain more brain disturbance before manifesting symptoms, whereas those with little cognitive reserve become symptomatic with more limited functional insults. An individual with more neuronal connections than another, for example, could sustain a substantially greater amount of neuronal dysfunction before becoming symptomatic. In degenerative disorders this would be translated into a decreased prevalence of the disease at a given age or a decreased severity of the disease for a given disease duration (figure 6).

Both genetic and environmental factors contribute to cognitive reserve. Genetic influences may influence synaptic density, which may have an effect on native intellectual ability, brain size and weight, and neuronal efficiency.[135] Environmental influences that affect cognitive reserve include education, age, and a history of head trauma. These also may be mediated in part by effects on synaptic density. Evidence in favor of the cognitive reserve hypothesis has been obtained from studies of delirium, human immunodeficiency virus encephalopathy, and vascular dementia as well as AD.[135-137] The effect is not disease-specific but occurs in different types of brain dysfunction.

In a study of aged patients with normal cognitive function who were found at autopsy to have many

neocortical plaques, Katzman et al.[136] noted that these asymptomatic patients had greater brain weights and larger numbers of neurons compared to age-matched controls. The reserve hypothesis predicts that these patients were able to sustain a degree of neuropathologic change consistent with early AD but had sufficient cognitive reserve to remain asymptomatic.

The threshold hypothesis predicts that AD patients with higher educational levels and greater intellectual capacity would have greater brain reserve and therefore could sustain substantially more cerebral dysfunction before becoming symptomatic. This prediction is consistent with the observations of Alexander et al.,[139] who found that estimates of premorbid intellectual ability were inversely correlated with the cerebral metabolism in AD patients (higher levels of education with lower levels of metabolism). Likewise, the presence of more severe brain disease at the time the symptom threshold is crossed in patients with higher cognitive function might predict that they would survive for a shorter period after becoming symptomatic and would experience more rapid cognitive decline or live for shorter periods of time after onset of symptoms; these predictions also have been confirmed.[140,141]

Figure 6 shows the interaction of the amyloid cascade with cognitive reserve and symptom thresholds. At one extreme is normal aging, with its very gradual production of Aβ such that dementia does not occur within the normal human lifespan. Patients with high educational levels are less likely to evidence AD, as observed empirically. At the other end of the spectrum are patients with Down syndrome, who have both reduced cognitive reserve and overproduction of Aβ, leading to occurrence of the dementia syndrome early in life. Next most severely affected are those with the inherited autosomal dominant forms of AD, in which Aβ is generated in unusual quantities, leading to the appearance of the dementia syndrome in late midlife. Next in order of appearance are patients who have normal Aβ production but are genetically at risk because of the presence of the ApoE-4 genotype or other disease-promoting factors. These lifetime trajectories interact with thresholds such that those who have a past history of head trauma, small heads, low intellectual function, or little education become symptomatic at earlier times than those with more propitious life circumstances. Although this model requires greater investigation and elaboration, it provides a framework for further research and an explanatory model for many observations; substantial evidence consistent with the model has accumulated.

Opportunities for treatment of AD. The amyloid cascade provides a means of conceptualizing therapeutic opportunities in AD. From a public health perspective, the increased longevity expected for the general population will increase the number of AD patients. Reducing the frequency of AD might be achieved by encouraging education and environmental enrichment strategies in childhood and by reducing the likelihood of head injury through use of seat belts, protective helmets, and better head protection in contact sports.

Currently available AD-specific therapies are of two types: symptomatic approaches based on enhancement of cholinergic function, and neuroprotective approaches utilizing antioxidant agents. Modest cognitive improvement has resulted from the administration of cholinomimetic agents. The available cholinesterase inhibitors tacrine and donepezil,[142-145] and emerging agents such as metrifonate, rivastigmine, eptastigmine, galantamine, and long-acting physostigmine, promise to provide practitioners with a range of cholinergic therapies.[146] In addition, cholinesterase inhibitors and cholinesterase receptor agonists have been shown to reduce the behavioral abnormalities often associated with AD.[147,148] Cholinesterase inhibitors improve function by enhancing residual cholinergic activity. Although the cholinergic disturbance may contribute to abnormal APP processing and cholinergic therapies may modulate this effect,[149] no definite disease-modifying effects of cholinergic enhancement therapy have been demonstrated.

Neuroprotective agents have been successfully employed to slow the progress of AD. Sano and colleagues[150] demonstrated that α-tocopherol or selegiline retarded the progress to major milestones in AD and reduced the loss of ability to perform activities of daily living. α-Tocopherol and selegiline did not improve existing symptoms.

Reduced Aβ production is an obvious therapeutic target in AD. In diseases such as Down syndrome, in which APP is overexpressed, regulation of the APP gene could be targeted by genetic therapies that would modulate gene activity. Increasing or amplifying the α-secretase pathway in favor of the β- and γ-secretase pathways might reduce the production of Aβ. Blockade of β- or γ-secretase offers another means of decreasing Aβ production. Finally, Aβ aggregation might be blocked through effects on ApoE-4 or through agents that block the conformational change of Aβ into the β-pleated sheet.[151]

As noted above, inflammatory pathways are activated in the neuritic plaques of AD, and NSAIDs may slow the progress and defer the onset of AD. Steroids or NSAIDs with acceptable side-effect profiles for chronic use in the elderly might represent useful approaches in the treatment of AD.[14] COX II inhibitors are under investigation.

Neuroprotective strategies also require more intensive study. Higher doses of α-tocopherol, use of other monoamine oxidase inhibitors, and exploration of other classes of antioxidant agents are warranted. As noted, the influx of calcium may be an integral part of neuronal injury in AD, and calcium-channel blockers should be tested for their potential therapeutic utility.[152] The possible role of excitatory amino acids in the mediation of cell death suggests that

blockade of *N*-methyl-D-aspartate (NMDA) receptors might be of benefit.[122]

Epidemiologic studies support a role for estrogen in the prevention and treatment of AD. The components of estrogen active in this regard, the role of conjugated estrogen preparation in AD, and the potential benefit of androgen manipulation in males require intensive investigation.

Nerve growth factor (NGF) prevents the degeneration of basal forebrain cholinergic neurons in experimental models of AD.[153] NGF does not cross the blood–brain barrier and must be administered intraventricularly or must be carried to the cell by implanted viral vectors. The latter approach has been used successfully in mice.[154] This suggests the possibility of transplantation therapy in AD, using viral vectors to transport NGF into the basal forebrain from a surgical implantation site.

Although—given the role of ACh in memory and cognition—almost all transmitter replacement research has focused on cholinergic treatment, treatment of other transmitter deficits in AD might be of benefit. Serotonin, norepinephrine, and somatostatin are reduced in the AD brain,[39] and development of therapeutic approaches using these transmitters and modulators is indicated.

Finally, psychotropic agents, including antipsychotics, antidepressants, anxiolytics, sedative hypnotics, and anti-agitation drugs, are available for treatment of the behavioral disturbances that occur in many AD patients.[155] These have not been subjected to rigorous double-blind studies, however, and their utility in AD requires more investigation.

Several general therapeutic principles emerge from consideration of the cascade approach to understanding the pathophysiology of AD. First, interventions that occur earlier in the cascade will have a greater opportunity to prevent symptoms and will have a greater effect on the panoply of events that characterize the ensuing AD process. Second, it is unlikely that agents sufficiently effective to interrupt the AD process entirely will be without substantial adverse effects on normal and necessary brain function. Therefore, it is more likely that synergistic partial effects at multiple steps will be required for treatment of AD. Poly-agent therapy can be anticipated. Third, agents that slow the progress of AD do not improve existing symptoms. Conversely, cholinergic agents that improve symptoms have little effect on the course of the illness. Therefore, treatment with cholinergic agents to improve symptoms, in conjunction with agents that slow the progress, represents a possible combination regimen. Fifth, because poly-agent therapy is anticipated, it is imperative for the interaction of agents to be carefully considered during the process of drug development. Agents may have interactions at the absorption, distribution, or site of action phases of therapy. In addition, combinations of side effects might be intolerable, or synergistic effects may produce enhanced benefits.

Comment. A number of fundamental questions arise from the approach to AD presented here. First, is AD a disease? A disease is defined as "a definite morbid process having a characteristic train of symptoms."[156] Early-onset AD readily lends itself to a disease interpretation. It is caused by an identified mutation that distinguishes it from other unaffected humans. It exhibits a characteristic course, produces relatively stereotyped symptoms, and causes disease at an unusually young age. It produces characteristic pathologic findings at autopsy. It is in the older age groups that application of the disease concept becomes more ambiguous. Neuritic plaques have been considered a part of the normal aging process because they are not infrequently found in nondemented elderly individuals at autopsy.[21] The distribution of neuropathologic changes in elderly nondemented individuals is identical to the pattern of distribution of the more abundant changes found in symptomatic individuals.[157-160] Moreover, long-term follow-up of asymptomatic individuals reveals that those who go on to dementia perform at a lower neuropsychological level during presymptomatic phases than do matched controls who do not proceed manifest dementia.[161,162] Whether late-onset sporadic AD should be viewed as an exaggeration of changes that occur at a low level of intensity in the course of normal aging, or as a separate and distinct disease process that occurs at very high frequencies in the elderly but is often asymptomatic at the time of death, is uncertain.

The second fundamental question is, can AD be prevented? AD potentially can be prevented by identification of individuals at high risk for the illness and treatment with agents that intervene in the pathogenetic cascade. Because most AD occurs late in life, slowing the onset of the illness by a single decade would be equivalent to preventing its occurrence. This strategy depends on identifying persons at risk for or in the presymptomatic phases of AD. Research as to how best to identify individuals early in the disease course is imperative.

Third, can AD be cured? Curing AD would require that symptomatic individuals would have sufficient improvement in symptoms to function normally. If cure means reversal of cell death, then cure is impossible with currently foreseeable technologies. If cure means becoming asymptomatic until death, then it is conceivable that patients detected in early stages and treated with agents producing substantial symptomatic relief could be cured.

Fourth, should treatment of AD be stage-specific? AD is conceptualized as at least a three-stage illness encompassing mild, moderate, and severe phases.[129] A reasonable therapeutic goal in AD is to prolong the period of functionality and to reduce the period of disability. From a pharmacotherapeutic point of view, this would entail the use of anti-amyloid, neuroprotective, and symptomatic therapies early in the course when functionality can be improved and maintained for a longer period of time. Prolongation

of the late phase of the illness may be undesirable for some patients and families. End-stage-specific therapy might emphasize agents aimed at increasing comfort and decreasing behavioral disturbances.

Summary. This is a particularly exciting time in the evolution of AD research. Sufficient information has accumulated to develop preliminary pathogenetic hypotheses and to provide a framework for therapeutic investigations. Treatments that improve the symptoms are available; neuroprotective therapies have been identified; and discovery of more disease-modifying agents is anticipated. Promising new agents are emerging and combination therapies appear promising. As the therapeutic armamentarium grows, increasingly effective and individualized therapies are envisioned. These advances offer hope that individuals with AD and their families can be spared the ravages of this tragic illness.

Acknowledgments

This project was supported by a National Institute on Aging Alzheimer's Disease Center Grant (AG10123), (P0 1 AG 12435, H. Vinters), an Alzheimer's Disease Research Center of California grant, and by the Sidell-Kagan Foundation, as well as by a grant from Bayer Corporation.

References

1. Cummings JL, Khachaturian Z. Alzheimer's disease: definitions and diagnostic criteria. In: Gauthier S, ed. Clinical diagnosis and management of Alzheimer's disease. London: Martin Dunitz, 1996:3–15.
2. McKhann G, Drachman DD, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–944.
3. Blacker D, Albert MS, Bassett SS, Go RCP, Harrell LE, Folstein MF. Reliability and validity of NINCDS-ADRDA criteria for Alzheimer's disease. The National Institute of Mental Health Genetics Initiative. Arch Neurol 1994;51:1198–1204.
4. Lopez OL, Swihart AA, Becker JT, et al. Reliability of NINCDS-ADRDA clinical criteria for the diagnosis of Alzheimer's disease. Neurology 1990;40:1517–1522.
5. Small GW, Mazziotta JC, Collins MT, et al. Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. JAMA 1995;273:942–947.
6. Langston JW, Tanner CM. Etiology of Parkinson's disease. In: Koller WC, ed. Handbook of Parkinson's disease. 2nd ed. New York: Marcel Dekker, 1992:369–381.
7. Gomez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997;41:17–24.
8. Terry RD. The pathology of Alzheimer's disease: numbers count [letter]. Ann Neurol 1997;41:7.
9. Dickson DW. The pathogenesis of senile plaques. J Neuropathol Exp Neurol 1997;56:321–339.
10. Landon M, Kidd M. The structure and protein chemistry of senile plaques and cerebrovascular amyloid. In: Davies DC, ed. Alzheimer's disease: towards an understanding of the aetiology and pathogenesis. London: John Libbey, 1989:65–75.
11. Mandybur TI, Chuirazzi CC. Astrocytes and the plaques of Alzheimer's disease. Neurology 1990;40:635–639.
12. Morris JC. Relationship of plaques and tangles to Alzheimer's disease phenotype. In: Goate AM, Ashall F, eds. Pathobiology of Alzheimer's disease. New York: Academic Press, 1995:191–223.
13. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
14. National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Consensus recommendations for the postmortem diagnosis of Alzheimers disease. Neurobiol Aging 1997;18(Suppl 4):51–52.
15. Aisen PS, Davis KL. Inflammatory mechanisms in Alzheimer's disease: implications for therapy. Am J Psychiatry 1994;151:1105–1113.
16. McGeer PL, Rogers J, McGeer EG. Neuroimmune mechanisms in Alzheimer disease patients. Alzheimer Dis Assoc Disord 1994;8:149–158.
17. Terry RD, Masliah E, Hansen LA. Structural basis of the cognitive alterations in Alzheimer disease. In: Terry RD, Katzman R, Bick KL, eds. Alzheimer disease. New York: Raven Press, 1994:179–196.
18. Braak H, Braak E. Pathology of Alzheimer's disease. In: Calne DB, ed. Neurodegenerative diseases. Philadelphia: WB Saunders, 1994:585–613.
19. Mega MS, Cummings JL, Fiorello T, Gornbein J. The spectrum of behavioral changes in Alzheimer's disease. Neurology 1996;46:130–135.
20. Duong T, De Rosa MJ, Poukens V, Vinters HV, Fisher RS. Neuronal cytoskeletal abnormalities in human cerebral cortical dysplasia. Acta Neuropathol 1994;87:493–503.
21. Crystal H, Dickson D, Fuld P, et al. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. Neurology 1988;38:1682–1687.
22. Schmidt ML, Lee VM-Y, Forman M, Chiu T-S, Trojanowski JQ. Monoclonal antibodies to the 10-kd protein reveal abundant Aβ-negative plaques throughout gray matter of Alzheimer's disease. Am J Pathol 1997;151:69–80.
23. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991;30:572–580.
24. Olichney JM, Hansen LA, Hofstetter R, Grundman M, Katzman R, Thal LJ. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol 1995;52:702–708.
25. Vinters HV, Wang ZZ, Secor DL. Brain parenchymal and microvascular amyloid in Alzheimer's disease. Brain Pathol 1996;6:179–195.
26. Ellis RJ, Olichney JM, Thal LJ, et al. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, part XV. Neurology 1996;46:1592–1596.
27. Lippa CF, Hamos JE, Smith TW, Pulaski-Salo D, Drachman DA. Vascular amyloid deposition in Alzheimer's disease: neither necessary nor sufficient for the local formation of plaques or tangles. Arch Neurol 1993;50:1088–1092.
28. Bornebroek M, Haan J, Maat-Schieman MLC, van Duinen SG, Roos RAC. Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): I-A review of clinical, radiologic and genetic aspects. Brain Pathol 1996;6:111–114.
29. Maat-Schieman MLC, van Duinen SG, Bornebroek M, Haan J, Roos RAC. Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): II-A review of histopathological aspects. Brain Pathol 1996;6:115–120.
30. Vinters HV. Cerebral amyloid angiopathy and Alzheimer's disease: two entities or one? [editorial]. J Neurol Sci 1992; 112:1–3.
31. Xu M, Shibayama H, Kobayashi H, et al. Granulovacuolar degeneration in the hippocampal cortex of aging and demented patients—a quantitative study. Acta Neuropathol (Berl) 1992;85:1–9.
32. Braak H, Braak E, Grundke-Iqbal I, Iqbal K. Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: a third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. Neurosci Lett 1986;65:351–355.
33. Davies P. Studies on the neurochemistry of central cholinergic systems in Alzheimer's disease. In: Katzman R, Terry RD, Bick KL, eds. Alzheimer's disease: senile dementia and related disorders. New York: Raven Press, 1978:453–459.
34. Hansen LA, DeTeresa R, Davies P, Terry RD. Neocortical morphometry, lesion counts, and choline acetyltransferase

levels in the age spectrum of Alzheimer's disease. Neurology 1988;38:48–54.
35. Wilcock GK, Esiri MM, Bowen DM, Smith CCT. Alzheimer's disease: correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J Neurol Sci 1982;57:407–417.
36. Schliebs R, Rossner S. Distribution of muscarinic acetylcholine receptors in the CNS. In: Stone TW, ed. CNS neurotransmitters and neuromodulators: acetylcholine. Boca Raton, FL: CRC Press, 1995:67–83.
37. Young AB, Penney JB Jr. Neurotransmitter receptors in Alzheimer disease. In: Terry RD, Katzman R, Bick KL, eds. Alzheimer disease. New York: Raven Press, 1994:293–303.
38. Mann DMA, Yates PO. Neurotransmitter deficits in Alzheimer's disease and in other dementing disorders. Hum Neurobiol 1986;5:147–158.
39. Palmer AM, Stratmann GC, Procter AW, Bowen DM. Possible neurotransmitter basis of behavioral changes in Alzheimer's disease. Ann Neurol 1988;23:616–620.
40. Whitehouse PJ. Neurotransmitter receptor alterations in Alzheimer disease: a review. Alzheimer Dis Assoc Disord 1987;1:9–18.
41. Hendriks L, van Broeckhoven C. The ssA 4 precursor protein gene and Alzheimer's disease. Eur J Biochem 1996;237:6–15.
42. Lendon CL, Ashall F, Goate AM. Exploring the etiology of Alzheimer disease using molecular genetics. JAMA 1997;277: 825–836.
43. Bird TD, Levy-Lahad E, Poorkaj P, et al. Wide range in age of onset for chromosome 1-related familial Alzheimer disease. Ann Neurol 1996;40:932–936.
44. Bird TD, Sumi SM, Nemens EJ, et al. Phenotypic heterogeneity in familial Alzheimer's disease: a study of 24 kindreds. Ann Neurol 1989;25:12–25.
45. Wisniewski KE, Wisniewski HM, Wen GY. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann Neurol 1985;17:278–282.
46. Govoni S, Bergamaschi S, Gasparini L, et al. Fibroblasts of patients affected by Down's syndrome oversecrete amyloid precursor protein and are hyporesponsive to protein kinase C stimulation. Neurology 1996;47:1069–1075.
47. Hardy J. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci 1997;20:154–159.
48. Mann DMA, Iwatsubo T, Cairns NJ, et al. Amyloid ss protein (Ass) deposition in chromosome 14-linked Alzheimer's disease: predominance of Ass42(43). Ann Neurol 1996;40:149–156.
49. Levy-Lahad E, Wasco W, Poorkaj P, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 1995;269:973–977.
50. Mann DMA, Iwatsubo T, Nochlin D, Sumi SM, Levy-Lahad E, Bird TD. Amyloid (Ass) deposition in chromosome 1-linked Alzheimer's disease: the Volga German families. Ann Neurol 1997;41:52–57.
51. Tsuang D, Kukull W, Sheppard L, et al. Impact of sample selection on ApoE e-4 allele frequency: a comparison of two Alzheimer's disease samples. J Am Geriatr Soc 1996;44:704–707.
52. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E Type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–923.
53. Gomez-Isla T, West HL, Rebeck GW, et al. Clinical and pathological correlates of apolipoprotein E e-4 in Alzheimer's disease. Ann Neurol 1996;39:62–70.
54. Olichney JM, Hansen LA, Galasko D, et al. The apolipoprotein E e4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. Neurology 1996;47:190–196.
55. Roses AD. Alzheimer's disease as a model of molecular gerontology. J NIH Res 1995;7:51–57.
56. Kang DE, Saitoh T, Chen X, et al. Genetic association of the low density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. Neurology 1997;49:56–61.
57. Payami H, Schellenberg GD, Zareparsi S, et al. Evidence for association of HLA-A2 allele with onset age of Alzheimer's disease. Neurology 1997;49:512–518.
58. Montoya SE, Aston CE, Dekosky SI, et al. Bleomycin hydrolase is associated with risk of sporatic Alzheimer's disease. Nat Genet 1998;18:211–217.
59. Breitner JCS, Silverman JM, Mohs RC, Davis KL. Familial aggregation in Alzheimer's disease: comparison of risk among relatives of early- and late-onset cases, and among male and female relatives in successive generations. Neurology 1988;38:207–212.
60. Mohs RC, Breitner JCS, Silverman JM, Davis KL. Alzheimer's disease: morbid risk among first-degree relative approximates 50% by 90 years of age. Arch Gen Psychiatry 1987;44:405–408.
61. Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a community population of older persons: higher than previously reported. JAMA 1989;262:2551–2556.
62. Jorm AF, Korten AE, Henderson AS. The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr Scand 1987;76:465–479.
63. Paykel ES, Brayne C, Huppert FA, et al. Incidence of dementia in a population older than 75 years in the United Kingdom. Arch Gen Psychiatry 1994;51:325–332.
64. Barclay L, Wolfson L. Normal aging: pathophysiologic and clinical changes. In: Barclay L, ed. Clinical geriatric neurology. Philadelphia: Lea & Febiger, 1993:13–20.
65. Powers RE. Neurobiology of Aging. In: Coffey CE, Cummings JL, eds. The American Psychiatric Press textbook of geriatric neuropsychiatry. Washington: American Psychiatric Press, 1994:35–69.
66. Rocca WA, Amaducci LA, Schoenberg BS. Epidemiology of clinically diagnosed Alzheimer's disease. Ann Neurol 1986; 19:415–424.
67. Rocca WA, Hofman A, Brayne C, et al. Frequency and distribution of Alzheimer's disease in Europe: a collaborative study of 1980–1990 prevalence findings. Ann Neurol 1991; 30:381–390.
68. Schoenberg BS, Anderson DW, Haerer AF. Severe dementia: prevalence and clinical features in a biracial US population. Arch Neurol 1985;42:740–743.
69. French LR, Schuman LM, Mortimer JA, Hutton JT, Boatman RA, Christians B. A case-control study of dementia of the Alzheimer type. Am J Epidemiol 1985;121:414–421.
70. Heyman A, Wilkinson WE, Stafford JA, Helms MJ, Sigmon AH, Weinberg T. Alzheimer's disease: a study of epidemiological aspects. Ann Neurol 1984;15:335–341.
71. Mayeux R, Ottman R, Tang MX, et al. Genetic susceptibility and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first-degree relatives. Ann Neurol 1993;33:494–501.
72. Broe GA, Henderson AS, Creasey H, et al. A case-control study of Alzheimer's disease in Australia. Neurology 1990;40: 1698–1707.
73. Chandra V, Kokmen E, Schoenberg BS, Beard CM. Head trauma with loss of consciousness as a risk factor for Alzheimer's disease. Neurology 1989;39:1576–1578.
74. Li G, Shen YC, Li YT, Chen CH, Zhau YW, Silverman JM. A case-control study of Alzheimer's disease in China. Neurology 1992;42:1481–1488.
75. Jordan BD, Kanik AB, Horwich MS, et al. Apolipoprotein E e-4 and fatal cerebral amyloid angiopathy associated with dementia pugilistica. Ann Neurol 1995;38:698–699.
76. Mayeux R, Ottman R, Maestre G, et al. Synergistic effects of traumatic head injury and apolipoprotein-e4 in patients with Alzheimer's disease. Neurology 1995;45:555–557.
77. Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. ss Amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. J Neurol Neurosurg Psychiatry 1994;57: 419–425.
78. Cassidy JW. Neuroanatomic and functional bases of behavior. In: Silver JM, Yudofsky SC, Hales RE, eds. Neuropsychiatry of traumatic brain injury. Washington: American Psychiatric Press, 1994:43–79.
79. Graves AB, Mortimer JA, Larson EB, Wenzlow A, Bowen JD, McCormick WC. Head circumference as a measure of cognitive reserve: association with severity of impairment in Alzheimer's disease. Br J Psychiatry 1996;169:86–92.
80. Mori E, Hirono N, Yamashita H, et al. Premorbid brain size as a determinant of reserve capacity against intellectual de-

cline in Alzheimer's disease. Am J Psychiatry 1997;154:18–24.
81. Schofield PW, Logroscino G, Andrews HF, Albert S, Stern Y. An association between head circumference and Alzheimer's disease in a population-based study of aging and dementia. Neurology 1997;49:30–37.
82. Schofield PW, Mosesson RE, Stern Y, Mayeux R. The age at onset of Alzheimer's disease and an intracranial area measurement: a relationship. Arch Neurol 1995;52:95–98.
83. Mortimer JA. Is Alzheimer's disease a lifelong illness? Risk factors for pathological and clinical disease. In: Heston LL, ed. Progress in Alzheimer's disease and similar conditions. Washington, DC: American Psychiatric Press, 1997:9–20.
84. Snowdon DA, Kemper SJ, Mortimer JA, Greiner LH, Wekstein DR, Markesbery WR. Linguistic ability in early life and cognitive function and Alzheimer's disease in late life: findings from the Nun Study. JAMA 1996;275:528–532.
85. Deary IJ, Caryl PG. Neuroscience and human intelligence differences. Trends Neurosci 1997;20:365–371.
86. Duara R, Barker WW, Lopez-Alberola R, et al. Alzheimer's disease: interaction of apolipoprotein E genotype, family history of dementia, gender, education, ethnicity, and age of onset. Neurology 1996;46:1575–1579.
87. Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer's disease. Proc Natl Acad Sci USA 1995;92:4725–4727.
88. Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nature Genet 1996;14:55–61.
89. LaDu MJ, Lukens JR, Reardon CA, Getz GS. Association of human, rat, and rabbit apolipoprotein E with beta-amyloid. J Neurosci Res 1997;49:9–18.
90. Yang DS, Smith JD, Zhou Z, Gandy SE, Martins RN. Characterization of the binding of amyloid-beta peptide to cell culture-derived native apolipoprotein E2, E3, and E4 isoforms and to isoforms from human plasma. J Neurochem 1997;68:721–725.
91. Wilson C, Mau T, Weisgraber KH, Wardell MR, Mahley RW, Agard DA. Salt bridge relay triggers defective LDL receptor binging by a mutant apolipoprotein. Structure 1997;2:713–718.
92. Breitner JCS, Gau BA, Welsh KA, et al. Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin study. Neurology 1994;44:227–232.
93. Canadian Study of Health and Aging. Risk factors for Alzheimer's disease in Canada. Neurology 1994;44:2073–2080.
94. Lucca U, Tettamanti M, Forloni G, Spagnoli A. Nonsteroidal antiinflammatory drug use in Alzheimer's disease. Biol Psychiatry 1994;36:850–853.
95. McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 1996;47:425–432.
96. Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997;48:626–632.
97. Rich JB, Rasmusson DX, Folstein MF, Carson KA, Kawas C, Brandt J. Nonsteroidal anti-inflammatory drugs in Alzheimer's disease. Neurology 1995;45:51–55.
98. Jouzeau JY, Terlain B, Abid A, Nedelec E, Netter P. Cyclooxygenase isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. Drugs 1997;53:563–582.
99. Chang JW, Coleman PD, O'Banion MK. Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease. Neurobiol Aging 1992;17:801–808.
100. Breitner JCS, Welsh KA, Helms MJ, et al. Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs. Neurobiol Aging 1995;16:523–530.
101. Paganini A, Henderson VW. Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 1994;140:256–261.
102. Ohkura T, Isse K, Akazawa K, Hamamoto M, Yaoi Y, Hagino N. Evaluation of estrogen treatment in female patients with dementia of the Alzheimer type. Endocrine J 1994;41:361–371.
103. Birge SJ. Is there a role for estrogen replacement therapy in the prevention and treatment of dementia? J Am Geriatr Soc 1996;44:865–870.
104. Luine VN. Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. Exp Neurol 1985;89:484–490.
105. Callahan CM, Hall KS, Hui SL, Musick BS, Unverzagt FW, Hendrie HC. Relationship of age, education, and occupation with dementia among a community-based sample of African Americans. Arch Neurol 1996;53:134–140.
106. Hill LR, Klauber MR, Salmon DP, et al. Functional status, education, and the diagnosis of dementia in the Shanghai survey. Neurology 1993;43:138–145.
107. Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of education and occupation on the incidence of Alzheimer's disease. JAMA 1994;271:1004–1010.
108. White L, Katzman R, Losonczy K, et al. Association of education with incidence of cognitive impairment in three established populations for epidemiologic studies of the elderly. J Clin Epidemiol 1994;47:363–374.
109. Zhang M, Katzman R, Salmon D, et al. The prevalence of dementia and Alzheimer's disease in Shanghai, China: impact of age, gender, and education. Ann Neurol 1990;27:428–437.
110. Fabrigoule C, Letenneur L, Dartigues JF, Zarrouk M, Commenges D, Barberger-Gateau P. Social and leisure activities and risk of dementia: a prospective longitudinal study. J Am Geriatr Soc 1995;43:485–490.
111. Katzman R. Education and the prevalence of dementia and Alzheimer's disease. Neurology 1993;43:13–20.
112. Cummings JL, Mega M. Alzheimer's disease: etiologies and pathogenesis. Consult Pharmacist 1996;11(suppl E):8–15.
113. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992;256:184–185.
114. Selkoe DJ. Physiological production of the ss-amyloid protein and the mechanism of Alzheimer's disease. Trends Neurol Sci 1993;16:403–409.
115. Selkoe DJ. Alzheimer's disease: genotypes, phenotype, and treatments. Science 1997;275:630–631.
116. Barrow CJ, Zagorski MG. Solution structures of ss peptide and its constituent fragments: relation to amyloid deposition. Science 1991;253:179–182.
117. Younkin SG. Evidence that Ass42 is the real culprit in Alzheimer's disease. Ann Neurol 1995;37:287–288.
118. Behl C, Davis JB, Cole GM, Schubert D. Vitamin E protects nerve cells from amyloid ss-protein toxicity. Biochem Biophys Res Commun 1992;186:944–950.
119. Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 1994;77:817–827.
120. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993;262:689–695.
121. Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36:747–751.
122. Beal MF. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 1992;31:119–130.
123. Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 1995;38:357–366.
124. Meda L, Cassatella MA, Szendrei GI, et al. Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature 1995;374:647–650.
125. Khachaturian ZS. Calcium hypothesis of Alzheimer's disease and brain aging. Ann NY Acad Sci 1994;747:1–11.
126. Mattson MP, Barger SW, Cheng B, Lieberburg I, Smith-Swintosky VL, Rydel RE. Beta-amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. Trends Neurosci 1993;16:409–414.
127. Bennett DA, Cochran EJ, Saper CB, Leverenz JB, Gilley DW, Wilson RS. Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's disease. Neurobiol Aging 1993;14:589–596.
128. Mann DM, Marcyniuk B, Yates PO, Neary D, Snowden JS. The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy. Neuropathol Appl Neurobiol 1988;4:177–195.

129. Cummings JL, Benson DF. Dementia: a clinical approach. 2nd ed. Boston: Butterworth-Heinemann, 1992.
130. Chase TN, Fedio P, Foster NL, Brooks R, Di Chiro G, Mansi L. Wechsler Adult Intelligence Scale performance: cortical localization by fluorodeoxyglucose F18-positron emission tomography. Arch Neurol 1984;41:1244–1247.
131. Haxby JV, Duara R, Grady CL, Cutler NR, Rapoport SI. Relations between neuropsychological and cerebral metabolic asymmetries in early Alzheimer's disease. J Cereb Blood Flow Metab 1985;5:193–200.
132. Sultzer DL. Behavioral syndromes in dementia: neuroimaging insights. Semin Clin Neuropsychiatry 1996;1:261–271.
133. Mortel KF, Meyer JS. Lack of postmenopausal estrogen replacement therapy and the risk of dementia. J Neuropsychiatry Clin Neurosci 1995;7:334–337.
134. Anderson K, Launer LJ, Ott A, Hoes AW, Breteler MMB, Hofman A. Do nonsteroidal anti-inflammatory drugs decrease the risk for Alzheimer's disease? The Rotterdam Study. Neurology 1995;45:1441–1445.
135. Stern RA, Silva SG, Chaisson N, Evans DL. Influence of cognitive reserve on neuropsychological functioning in asymptomatic human immunodeficiency virus-1 infection. Arch Neurol 1996;53:148–153.
136. Gorelick PB, Brody J, Cohen D, et al. Risk factors for dementia associated with multiple cerebral infarcts: a case-control analysis in predominantly African-American hospital-based patients. Arch Neurol 1993;50:714–720.
137. Reyes-Ortiz CA. Delirium, dementia and brain reserve [letter]. J Am Geriatr Soc 1997;45:778–783.
138. Katzman R, Terry R, De Teresa R, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. Ann Neurol 1988;23:138–144.
139. Alexander GE, Furey ML, Grady CL, et al. Association of premorbid intellectual function with cerebral metabolism in Alzheimer's disease: implications for the cognitive reserve hypothesis. Am J Psychiatry 1997;154:165–172.
140. Stern Y, Tang MX, Denaro J, Mayeux R. Increased risk of mortality in Alzheimer's disease patients with more advanced educational and occupational attainment. Ann Neurol 1995;37:590–595.
141. Teri L, McCurry SM, Edland SD, Kukull WA, Larson EB. Cognitive decline in Alzheimer's disease: a longitudinal investigation of risk factors for accelerated decline. J Gerontol [A] 1995;50A:M49–55.
142. Davis KL, Thal LJ, Gamzu ER, et al. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. N Engl J Med 1992;327:1253–1259.
143. Farlow M, Gracon SI, Hershey LA, Lewis KW, Sadowsky CH, Dolan-Ureno J, for the Tacrine Study Group. A controlled trial of tacrine in Alzheimer's disease. JAMA 1992;68: 2523–2529.
144. Knapp MJ, Knopman DS, Solomon PR, Pendlebury WW, Davis CS, Gracon SI, for the Tacrine Study Group. A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. JAMA 1994;271:985–991.
145. Rogers SL, Friedhoff LT, and the Donepezil Study Group. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US multicentre, randomized, double-blind, placebo-controlled trial. Adapted from Dementia 1996;7:293–303.
146. Tariot PN, Schneider L. Contemporary treatment approaches to Alzheimer's disease. Consult Pharm (suppl E):16–24.
147. Bodick NC, Offen WW, Levey AI, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. Arch Neurol 1997;54:465–473.
148. Cummings JL, Kaufer D. Neuropsychiatric aspects of Alzheimer's disease: the cholinergic hypothesis revisited. Neurology 1996;47:876–883.
149. Wallace WC, Bragin V, Robakis NK, et al. Increased biosynthesis of Alzheimer amyloid precursor protein in the cerebral cortex of rats with lesions of the nucleus basalis of Meynert. Mol Brain Res 1991;10:173–178.
150. Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. N Engl J Med 1997;336:1216–1222.
151. Whyte S, Beyreuther K, Masters CL. Rational therapeutic strategies for Alzheimer's disease. In: Calne DB, ed. Neurodegenerative diseases. Philadelphia: WB Saunders, 1994: 647–664.
152. Tollefson GD. Short-term effects of the calcium channel blocker nimodipine (Bay-e-9736) in the management of primary degenerative dementia. Biol Psychiatry 1990;27:1133–1142.
153. Koliatsos VE, Clatterbuck RE, Nauta HJW, et al. Human nerve growth factor prevents degeneration of basal forebrain cholinergic neurons in primates. Ann Neurol 1991;30:831–840.
154. Rosenberg MB, Friedmann T, Robertson RC, et al. Grafting genetically modified cells to the damaged brain: restorative effects of NGF expression. Science 1988;242:1575–1578.
155. Cummings JL. Neuropsychiatric assessment and intervention in Alzheimer's disease. Int Psychogeriatr 1996;8(suppl 1):25–30.
156. Dorland's Illustrated Medical Dictionary. 24th ed. Philadelphia: WB Saunders, 1965.
157. Arriagada PV, Marzloff K, Hyman BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 1992;42:1681–1688.
158. Giannakopoulos P, Hof PR, Giannakopoulos A-S, Herrmann FR, Michel J-P, Bouras C. Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of very old patients. Arch Neurol 1995;52:1150–1159.
159. Morris JC, Storandt M, McKeel DW Jr, et al. Cerebral amyloid deposition and diffuse plaques in "normal" aging: evidence for presymptomatic and very mild Alzheimer's disease. Neurology 1996;46:707–719.
160. Ulrich J. Alzheimer changes in nondemented patients younger than sixty-five: possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. Ann Neurol 1985;17:273–277.
161. Linn RT, Wolf PA, Bachman DL, et al. The 'preclinical phase' of probable Alzheimer's disease: a 13-year prospective study of the Framingham Cohort. Arch Neurol 1995;2:485–490.
162. Masur DM, Sliwinski M, Lipton RB, Blau AD, Crystal HA. Neuropsychological prediction of dementia and the absence of dementia in healthy elderly persons. Neurology 1994;44: 1427–1432.

EXHIBIT C

ELSEVIER

Journal of Chemical Neuroanatomy 16 (1998) 35–41

Journal of CHEMICAL NEUROANATOMY

What are the facts and artifacts of the pathogenensis and etiology of Alzheimer disease?

Mark A. Smith, George Perry *

Case Western Reserve University, Institute of Pathology, 2085 Adelbert Road, Cleveland, OH 44106, USA

Received 11 May 1997; accepted 27 September 1998

Abstract

Over the past decade, an increased clinical awareness, together with advances in biochemical, cellular, and molecular analyses, have catapulted the study of Alzheimer disease to the forefront of biomedical research. During this time, a great number of theories, regarding disease pathogenesis, have come and gone but several have persisted. Here, we critically evaluate these theories in an attempt to delineate the facts from the artifacts.

1. Introduction

The pace of research on Alzheimer disease (AD) has grown exponentially over the past decade as reflected in the increased number of publications (Fig. 1). Yet, by measures of our understanding of the pathogenesis of the disease, we are not much closer to a mechanistic view of its etiology than we were over two decades ago, when many of the ideas directing this research were initiated. Obviously, the study of a multisystem brain disorder with genetic and sporadic factors and involving essentially half of the aged population is complex (Smith, 1998). Indeed, an emerging view is that AD is more than a single etiological entity, but rather part of the spectrum of degenerative diseases affecting the aged that share common etiologies. Here, we consider where our knowledge of AD stands, the facts, and those aspects, which often led to the investigator-induced interpretation, that are the artifacts of AD.

2. What is Alzheimer disease?

One of the changes in our understanding of AD from a public health standpoint was the realization that AD was not a disease restricted to middle-aged patients, as described in the original study by Alois Alzheimer (Alzheimer, 1907). In studies of the elderly, the lesions that Alzheimer described in a woman in her 50's were shown to be identical and correlated to the extent of senile dementia in the aged (e.g. Roth et al., 1966, 1967; Tomlinson et al., 1970). In fact, as discussed later, age is the greatest risk factor for the development of AD with an exponential increase in disease incidence beginning at 65 years (Fig. 2).

3. Diagnosis

The diagnosis of AD is not based on defined qualitative features but, rather, is differentiated from normal aging based on the number of senile plaques in the cortical extracellular space and neurofibrillary tangles within vulnerable neurons. Since the disease is defined by these lesions, it is perhaps not surprising that they are also the focus of research efforts (Roses, 1994). Yet in formulating the diagnostic and consequent disease

* Corresponding author. Tel.: +1 216 3682488; fax: +1 216 3688964; e-mail: gxp7@po.cwru.edu

0891-0618/98/$ - see front matter
PII S0891-0618(98)00061-1

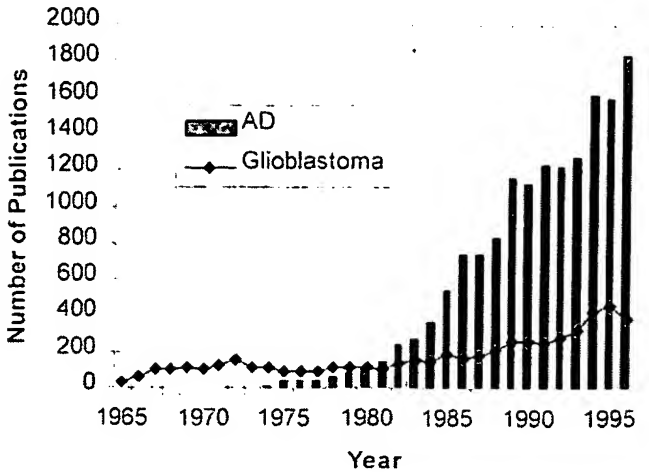


Fig. 1. Comparison of the number of publications per year related to two important human brain diseases 'Alzheimer disease' and 'glioblastoma', based on analysis of entries in Medline with those key words in the title or abstract. Note the number of articles related to Alzheimer disease has enjoyed an exponential increase since the mid 1970s while the number of articles related to glioblastoma has increased more modestly.

description based on the pathological lesions, the prior conceptual basis that dementia stemmed from vascular disease was completely excluded (Khachaturian, 1985; Mirra et al., 1991). Indeed, cases of dementia displaying cerebrovascular disease, including those with considerable numbers of neurofibrillary tangles and senile plaques, are not considered as AD. While at first appearance, this leaves AD as a well-defined entity, it obviously also precludes any understanding of the interaction between factors affecting the vasculature and AD. Therefore, while dementia due to vascular compromise underlies at least 20% of cases of clinical dementia (Perry and Smith, 1998), less than 1% of pathologically-defined AD cases show major vascular involvement. The exclusion of major vascular involvement from a diagnosis of AD obviously biases efforts to establish linkage between AD and a number of other age-related conditions (Smith et al., 1996a; Grant, 1997; Smith et al., 1997a). Therefore, when a genetic link between AD and apolipoprotein E was discovered (Corder et al., 1993), the well-established connection between apolipoprotein E and vascular disease was never proposed as a biological or pathological mechanism. Instead, pathways related to defining links to neurofibrillary tangles and senile plaques were pursued (Strittmatter et al., 1993; Whitson et al., 1994; Richey et al., 1995). However, if cases with vascular complications are included in AD, cerebrovascular atherosclerosis is actually found to be a major risk factor for AD and the major predictor of dementia in cases followed prospectively (Hofman et al., 1997; Snowdon et al., 1997).

4. Genetics

Genes on chromosomes 1 (Presenilin 2), 14 (Presenilin 1), 19 (apolipoprotein E) and 21 (β protein precursor) are all genetically linked to AD and, with the exception of apolipoprotein E which is considered a risk factor, are responsible for the majority of AD cases with autosomal dominant inheritance [reviewed in Selkoe (1997)]. At most, 5% of all cases of AD show a clear autosomal inheritance. The implications of these genetic findings are fiercely debated and one of the major thrusts is an argument in favor of a primary role for amyloid-β since alterations in amyloid-β metabolism are affected by all of the genetic mutations [reviewed in Selkoe (1997)]. Indeed, mutations in â-protein precursor or presenilins 1 and 2 either lead to increased amyloid-β and/or greater formation of the 42 amino acid form of amyloid-β. Together these promote amyloid-β fiber formation by, respectively, increasing amyloid-β concentration (mass action) or lowering amyloid-β solubility (critical concentration). Nonetheless, the importance of amyloid aggregation, including amyloid-β_{1-42}, in initiating AD stems from concepts learned from in vitro experiments, while in vivo studies instead suggest that amyloid-β deposits lie in morphological proximity to normal structures such as neurons (Allsop et al., 1989) and vessels (Kawai et al., 1990). This indicates that the morphogenesis of amyloid-β polymers, like physiological polymers such as microtubules which form on centrioles, are initially deposited on non-amyloid-β containing structures, i.e. amyloid-β in vivo is unlikely to be self-nucleated. In this vein, amyloid-β interactions with heparan sulfate proteoglycans (Snow et al., 1990), τ of neurofibrillary tangles (Smith et al., 1995a), gangliosides (Choo-Smith et al., 1997) or other components undoubtedly play a key role in aggregation (Perry et al., 1991). Therefore, while amyloid-β_{1-42} may form the initial amyloid-β deposits found in AD, the mechanisms for amyloid-β deposition may be quite distinct from those governing free amyloid-β in a test tube. One only needs to look at a senile plaque in the microscope to appreciate its structural complexity involving microglia, astrocytes and neurons as well as a host of protein-associated elements. Which factor is essential for the deposition of amyloid-β in the brain is not yet established, however, the lack of amyloid-β deposits in Down syndrome until late childhood, even though amyloid-β levels are high throughout life (Teller et al., 1996), suggests that age-related changes play a pivotal role in determining whether amyloid-β remains soluble or is deposited.

The extent and intensity of research into amyloid-β is somewhat surprising in light of the fact that amyloid-β deposits show only a weak relationship to dementia, and there is no evidence for a primary role for amyloid-β in the disease. Further, although amyloid-β polymer-

ization is linked to increased toxicity in vitro (Sayre et al., 1997), the relationship in vivo is more complex. Injection of isolated senile plaques into rat brains (Frautschy et al., 1992) or incubation of primary cultures of rat neurons with amyloid-β (or isolated senile plaques, Canning et al., 1993; DeWitt et al., 1998) shows that, under circumstances closest to physiological, amyloid-β is not toxic. Consistent with these findings, transgenic mice with massive amyloid-β deposits (Hsiao et al., 1996) or normal cases of aging with abundant diffuse senile plaques show no neuronal loss arguing against a direct role for amyloid-β in neuronal death and not supporting the amyloid cascade hypothesis (Hardy and Higgins, 1992). Therefore, efforts to link the genetic factors to amyloid-β metabolism alone seem to miss the point that amyloid-β, as well as other markers of the disease, are just as likely to be 'protective' as they are 'toxic' responses to an underlying etiology. Simply put, at this time, all we know from the markers is that they are factors that could play a role in the disease, no more, no less. Instead, age is the major factor involved in the pathogenesis of AD and is more than the time required for amyloid-β fibrillogenesis. The brain is not a beaker of a homogenous salt solution waiting to crystallize; anabolic as well as catabolic processes, which undoubtedly change with advancing age, must be involved in amyloid deposition.

5. Pathological lesions and neuronal viability

While early studies suggested that the degree of cognitive impairment in AD correlated with the numbers of senile plaques (Blessed et al., 1968) and neurofibrillary tangles (Wilcock and Esiri, 1982), more recent studies indicate that, despite increasing dementia, the number of lesions often remain stable (Mann et al., 1988; Bennett et al., 1993). Furthermore, considerable pathological involvement is often found in the absence of cognitive impairment. Therefore, despite being considered as the pathological hallmarks of the disease, the number of senile plaques or neurofibrillary tangles is not a particularly good indicator of the severity of the disease process. Changes that correlate best with the decline in cognitive function include loss of pyramidal neurons (Mann et al., 1988) and indicators of neuronal connectivity such as synapse loss or neuritic alterations (Masliah et al., 1989, 1990; Scheff et al., 1990) suggesting that compromised neuronal function precedes and is more widespread than lesion formation and neuronal death. While synapse loss may be the most proximal cause of cognitive loss, its biological basis is most likely neuritic abnormalities. The importance of neuritic pathology is indicated by its occurrence in the earliest forms of amyloid-β deposition found in normal aging (Cras et al., 1991; Rifenburg and Perry, 1995; Morris et al., 1996) and Down syndrome (Murphy et al., 1990). Furthermore, since amyloid-β deposits, whether diffuse or classical, contain both amyloid-β fibrils and neuritic abnormalities, there is no substantial evidence supporitng the amyloid cascade hypothesis that toxicity results from amyloid-β fibrillogenesis (Hardy and Higgins, 1992). Instead, there is a relationship that suggests accumulation of β-protein precursor in dystrophic neurites may be the source of the amyloid-β found in senile plaques (Perry et al., 1988; Perry and Smith, 1993; Praprotnik et al., 1996a). Such neuritic and synaptic abnormalities may be due to effects on membrane properties including fluidity, replenishment, integrity and calcium flux (Bosman et al., 1991; Nitsch et al., 1992; Roth et al., 1995; Praprotnik et al., 1996a) caused by amyloid-β protein toxicity [reviewed in Iversen et al. (1995)], axonal transport deficiencies (Suzuki and Terry, 1967; Terry, 1996; Praprotnik et al., 1996b; Smith and Perry, 1997) or oxidative stress (Smith et al., 1991; Benzi and Moretti, 1995; Hensley et al., 1995; Smith et al., 1995b; Markesbery, 1997).

In AD, the most studied neuronal response is the hyperphosphorylation of a variety of cellular proteins including τ and neurofilament proteins (Sternberger et al., 1985; Saitoh et al., 1991). The effects of phosphorylation are proposed as key features leading to the formation of insoluble protein aggregates and neurofibrillary tangles. Initial proposals of specific increases in kinases or reduction in phosphatases (Trojanowski et al., 1993) have all been fraught with interpretative problems because the same changes in τ phosphorylation can be found normally, including in development (Goedert et al., 1993). Further, the biochemical properties of neurofibrillary tangles are unchanged with phosphate removal (Smith et al., 1996b) and τ phosphorylation is not required for its incorporation into neurofibrillary tangles (Bondareff et al., 1995). Further, while phosphorylation controls the function of τ to stabilize microtubules (Baudier and Cole, 1987), possibly diverting it to competing functions (Smith et al., 1995a), the underlying reason for increased phosphorylation has not been addressed. Intriguingly, oxidative stress activates the signal transduction cascades between the ERK or JNK pathways, respectively, deciding whether to enter the cell cycle or die by apoptosis. Neurons in AD appear to evade death by entering the former through activation of ERK (Smith and Perry, unpublished) with consequent upregulation of cell cycle related proteins (McShea et al., 1997). Therefore, increased phosphorylation may simply be a response to pathogenic stimuli, such as neuronal oxidative damage, rather than a fundamental change. Indeed, it appears that neurons attempt to cope with chronic insults of aging by using the same responses initiated by acute insults, such as head trauma, including, but not limited to, increased β-protein precursor

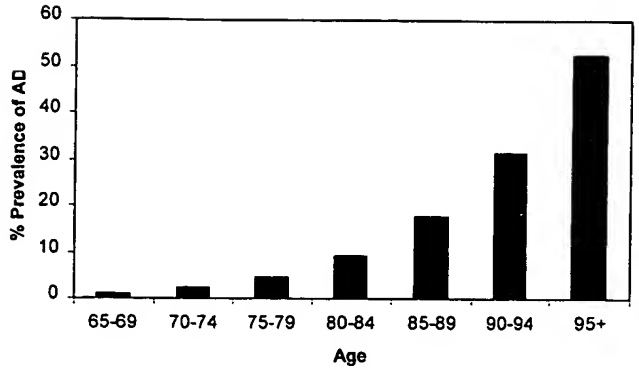


Fig. 2. Data from the US Government Accounting Office-Health and Human Services analysis (1998) of the prevalence of AD in the aged population.

expression (Roberts et al., 1991), cytoskeletal phosphorylation (Kanayama et al., 1996) and activation of cell cycle-related proteins (Van Lookeren Campagne and Gill, 1998).

Oxidative damage occurs not only to the lesions, which are extensively modified by oxidative crosslinks (Smith et al., 1996b), but, more significantly, is also found in the cell bodies of all vulnerable neurons in AD, whether or not they contain lesions. The source of damage must lie close at hand, because reactive oxygen can diffuse only short distances within tissue. Oxidative damage is therefore not only independent of lesions, but occurs prior to the earliest described cytopathology associated with neurofibrillary tangles (Smith et al., 1996c, 1997b). The relative uniformity of oxidative damage for all the neurons within a vulnerable population suggests there are factors that coordinate the response of involved neurons. Among the possibilities, amyloid-β may play a key role since transgenic mice overexpressing amyloid-β as well as cases of Down syndrome, known to have elevated amyloid-β even as fetuses, show oxidative damage similar to AD (Odetti et al., 1998; Smith et al., 1998a). Another possibility is that nitric oxide, from neuronal or microglia inflammation, can react with superoxide produced in neurons to form peroxynitrite and, in this regard, peroxynitrite-mediated damage also affects all vulnerable neurons in AD (Smith et al., 1997b).

6. Aluminum

Aluminum induces neuronal neurofibrillary changes, probably by interfering with axonal transport of neurofilaments (Bizzi et al., 1984; Troncoso et al., 1986), leading to the suggestion that aluminum may be an environmental toxin that triggers AD (Klatzo et al., 1965; Terry and Peña, 1965). Indeed, the correlation between endogenous levels of aluminum and increased incidence of AD supports such a notion (Martyn et al., 1989; McLachlan et al., 1996). Further, increased concentrations of aluminum in the brains of cases of AD (Crapper et al., 1973, 1976; Perl and Brody, 1980; Candy et al., 1986), while controversial (McDermott et al., 1979; Landsberg et al., 1992), suggested that aluminum might be an important pathological or etiological factor. Nonetheless, more recent studies have suggested that aluminum is simply a marker of a fundamental alteration in metal metabolism associated with iron accumulation (Good et al., 1992; Smith et al., 1997c). Indeed, since aluminum and iron are both transported and stored respectively by transferrin and ferritin, the findings with aluminum may simply mark alterations in iron metabolism (Smith et al., 1998b) that also result in accumulation of redox-active iron in vulnerable neurons (Smith et al., 1997c, Smith et al., unpublished observations). Iron, in contrast to aluminum, has complex biological functions that, as stated earlier, are altered with the onset of AD and are highly integrated with oxidative balance (Smith et al., 1997c).

7. Aging

Age represents, by far, the single greatest risk factor for development of AD (Fig. 2) and, even in genetically-predisposed individuals, the disease rarely occurs prior to age 50. Therefore, regardless of whether or not one is genetically predisposed, aging is an essential factor in AD, strongly suggesting that an age-related process is involved in the development of the disease.

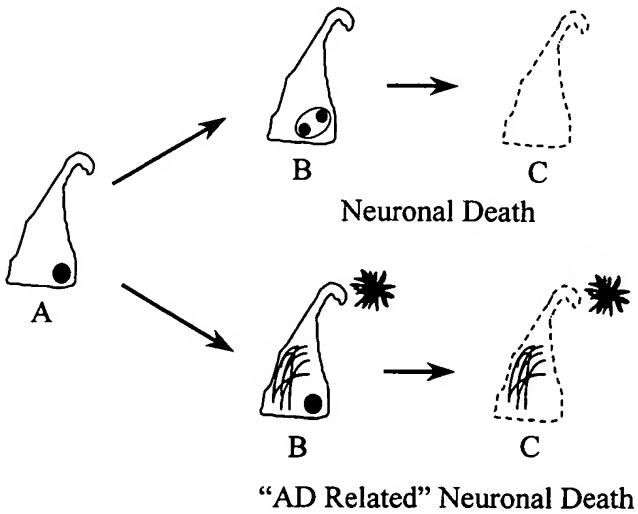


Fig. 3. In 'normal' neuronal death, neurons (A) proceed through apoptotic (B) or necrotic changes prior to death (C). In contrast, in AD, neurons vulnerable to death, live for years even though they show extensive neurofibrillary tangle and senile plaque involvement (B) prior to death (C) marked by the remaining extracellular neurofibrillary tangle.

This age-related penetrance is not restricted to AD and is also a risk factor in a number of other chronic diseases including other neurodegenerative diseases, cancer, atherosclerosis, arthritis and emphysema indicating the possibility that there may be common etiologies among degenerative diseases (Grant, 1997). So while discussing AD as a defined disease entity with a single pathogenesis, data also supports AD as a part of the repertoire of age-related compensations that maintain 'normal' functioning during aging. The high concordance of AD and other age-related diseases (Grant, 1997) certainly suggests that differentials in age-related compensations are central to development of AD and other degenerative diseases. Viewed in this light, genetics is a window, to unlock the compensations that maintain normal function. Understanding the role of these compensations will show if the cellular changes associated with formation of neurofibrillary tangles and senile plaques are responses to aging that preserves neuronal life (Fig. 3) as an alternative to apoptotic death. Albeit, a response that eventually leaves neuronal structures so modified, they cannot maintain neuronal physiological function in information transfer.

8. Conclusions

Despite intense research, the pathogenesis of AD still remains enigmatic and is likely to remain so for the foreseeable future. The primary responsibility is that while AD may have a single cellular pathogenesis, the etiology is heterogeneous. Nonetheless, as research drifts away from the study of genetic and physiology focused on lesions to more fundamental cellular processes, such as oxidative imbalance that change with advancing age, an appreciation of the primary pathogenesis will emerge and possibly lead to the development of effective therapeutic strategies.

Acknowledgements

Research in the authors' laboratories is supported by the National Institutes of Health, the American Health Assistance Foundation and the Alzheimer's Association.

References

Allsop, D., Haga, S.-I., Haga, C., Ikeda, S.-I., Mann, D.M.A., Ishii, T., 1989. Early senile plaques in Down's syndrome brains show a close relationship with cell bodies of neurons. Neuropathol. Appl. Neurobiol. 15, 531–542.

Alzheimer, A., 1907. In: Rottenberg, D.A., Hochberg, F.H. (Eds.), Allg. Z. Psychiatrie Psychisch-Gerichtliche Medizin 64, 146–148. Translated in Neurological Classics in Modern Translation, 1977. Hafner Press, New York, pp. 41–43.

Baudier, J., Cole, R.D., 1987. The phosphorylation of the microtubule-associated tau. In: Perry, G. (Ed.), Advances in Behavioral Biology 34: Alterations in the Neuronal Cytoskeleton in Alzheimer Disease. Plenum Press, New York, pp. 25–32.

Bennett, D.A., Cochran, E.J., Saper, C.B., Leverenz, J.B., Gilley, D.W., Wilson, R.S., 1993. Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's disease. Neurobiol. Aging 14, 589–596.

Benzi, G., Moretti, A., 1995. Are reactive oxygen species involved in Alzheimer's disease? Neurobiol. Aging 16, 661–674.

Bizzi, A., Crane, R.C., Autilio-Gambetti, L., Gambetti, P., 1984. Aluminum effect on slow axonal transport: a novel impairment of neurofilament transport. J. Neurosci. 4, 722–731.

Blessed, G., Tomlinson, B.E., Roth, M., 1968. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Brit. J. Psychiat. 114, 797–811.

Bondareff, W., Harrington, C.R., Wischik, C.M., Hauser, D.L., Roth, M., 1995. Absence of abnormal hyperphosphorylation of tau in intracellular tangles in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 54, 657–663.

Bosman, G.J., Bartholomeus, I.G., de Grip, W.J., 1991. Alzheimer's disease and cellular aging: membrane-related events as clues to primary mechanisms. Gerontology 37, 95–112.

Candy, J.M., Oakley, A.E., Klinowski, J., et al., 1986. Aluminosilicates and senile plaque formation in Alzheimer's disease. Lancet 1, 354–357.

Canning, D.R., McKeon, R.J., DeWitt, D.A., Perry, G., Wujek, J.R., Frederickson, R.C.A., Silver, J., 1993. β-amyloid of Alzheimer's disease induces reactive gliosis that inhibits axonal outgrowth. Exp. Neurol. 124, 289–298.

Choo-Smith, L.P., Garzon-Rodriguez, W., Glabe, C.G., Surewicz, W.K., 1997. Acceleration of amyloid fibril formation by specific binding of Aβ-(1-40) peptide to ganglioside-containing membrane vesicles. J. Biol. Chem. 272, 22987–22990.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., et al., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.

Crapper, D.R., Krishnan, S.S., Dalton, A.J., 1973. Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. Science 180, 511–513.

Crapper, D.R., Krishnan, S.S., Quittkat, S., 1976. Aluminium, neurofibrillary degeneration and Alzheimer's disease. Brain 99, 67–80.

Cras, P., Kawai, M., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., Perry, G., 1991. Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proc. Natl. Acad. Sci. USA 88, 7552–7556.

DeWitt, D.A., Perry, G., Cohen, M., Doller, C., Silver, J., 1998. Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. Exp. Neurol. 149, 329–340.

Frautschy, S.A., Cole, G.M., Baird, A., 1992. Phagocytosis and deposition of vascular beta-amyloid in rat brains injected with Alzheimer beta-amyloid. Am. J. Pathol. 140, 1389–1399.

Goedert, M., Jakes, R., Crowther, R.A., et al., 1993. The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. Proc. Natl. Acad. Sci. USA 90, 5066–5070.

Good, P.F., Perl, D.P., Bierer, L.M., Schmeidler, J., 1992. Selective accumulation of aluminum and iron in the neurofibrillary tangles of Alzheimer's disease: a laser microprobe (LAMMA) study. Ann. Neurol. 31, 286–292.

Grant, W.B., 1997. Dietary links to Alzheimer's disease. Alz. Dis. Rev. 2, 42–55.

Hardy, J.A., Higgins, G.A., 1992. Alzheimer's disease: the amyloid cascade hypothesis. Science 256, 184–185.

Hensley, K., Hall, N., Subramaniam, R., et al., 1995. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J. Neurochem. 65, 2146–2156.

Hofman, A., Ott, A., Breteler, M.M., et al., 1997. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam study. Lancet 349, 151–154.

Hsiao, K., Chapman, P., Nilsen, S., et al., 1996. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 274, 99–102.

Iversen, L.L., Mortishire-Smith, R.J., Pollack, S.J., Shearman, M.S., 1995. The toxicity in vitro of β-amyloid protein. Biochem. J. 331, 1–16.

Kanayama, G., Takeda, M., Niigawa, H., et al., 1996. The effects of repetitive mild brain injury on cytoskeletal protein and behavior. Methods Findings Exp. Clin. Pharmacol. 18, 105–115.

Kawai, M., Kalaria, R.N., Harik, S.I., Perry, G., 1990. The relationship of amyloid plaques to cerebral capillaries in Alzheimer's disease. Am. J. Pathol. 137, 1435–1446.

Khachaturian, Z.S., 1985. Diagnosis of Alzheimer's disease. Arch. Neurol. 42, 1097–1105.

Klatzo, I., Wisniewski, H.M., Streicher, E., 1965. Experimental production of neurofibrillary degeneration. J. Neuropathol. Exp. Neurol. 24, 187–199.

Landsberg, J.P., McDonald, B., Watt, F., 1992. Absence of aluminium in neuritic plaque cores in Alzheimer's disease. Nature 360, 65–68.

Mann, D.M.A., Marcyniuk, B., Yates, P.O., Neary, D., Snowden, J.S., 1988. The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy. Neuropathol. Appl. Neurobiol. 14, 177–195.

Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Rad. Biol. Med. 23, 134–147.

Martyn, C.N., Barker, D.J., Osmond, C., Harris, E.C., Edwardson, J.A., Lacey, R.F., 1989. Geographical relation between Alzheimer's disease and aluminum in drinking water. Lancet 1, 59–62.

Masliah, E., Terry, R.D., DeTeresa, R.M., Hansen, L.A., 1989. Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease. Neurosci. Lett. 103, 234–239.

Masliah, E., Terry, R.D., Mallory, M., Alford, M., Hansen, L.A., 1990. Diffuse plaques do not accentuate synapse loss in Alzheimer's disease. Am. J. Pathol. 137, 1293–1297.

McDermott, J.R., Smith, A.I., Iqbal, K., Wisniewski, H.M., 1979. Brain aluminum in aging and Alzheimer disease. Neurology 29, 809–814.

McLachlan, D.R., Bergeron, C., Smith, J.E., Boomer, D., Rifat, S.L., 1996. Risk for neuropathologically confirmed Alzheimer's disease and residual aluminum in municipal drinking water employing weighted residential histories. Neurology 46, 401–405.

McShea, A., Harris, P.L.R., Webster, K.R., Wahl, A.F., Smith, M.A., 1997. Abnormal expression of the cell cycle regulators p16 and CDK4 in Alzheimer's disease. Am. J. Pathol. 150, 1933–1939.

Mirra, S.S., Heyman, A., McKeel, D., et al., 1991. The consortium to establish a registry for Alzheimer's disease (CERAD): Part II, standardization of the neuropathologic assessment of Alzheimers disease. Neurology 41, 479–486.

Morris, J.C., Storandt, M., McKeel, D.W.J., Rubin, E.H., Price, J.L., Grant, E.A., Berg, L., 1996. Cerebral amyloid deposition and diffuse plaques in 'normal' aging: evidence for presymptomatic and very mild Alzheimer's disease. Neurology 46, 707–719.

Murphy, G.M.J., Eng, L.F., Ellis, W.G., Perry, G., Meissner, L.C., Tinklenberg, J.R., 1990. Antigenic profile of plaques and neurofibrillary tangles in the amygdala in Down's syndrome: a comparison with Alzheimer's disease. Brain Res. 537, 102–108.

Nitsch, R.M., Blusztajn, J.K., Pittas, A.G., Slack, B.E., Growdon, J.H., Wurtman, R.J., 1992. Evidence for a membrane defect in Alzheimer disease brain. Proc. Natl. Acad. Sci. USA 89, 1671–1675.

Odetti, P., Angelini, G., Dapino, D., et al., 1998. Early glycoxidation damage in brains from Down's syndrome. Biochem. Biophys. Res. Comm. 243, 849–851.

Perl, D.P., Brody, A.R., 1980. Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science 208, 297–299.

Perry, G., Lipphardt, S., Mulvihill, P., Kancherla, M., Mijares, M., Gambetti, P., Sharma, S., Maggiora, L., Cornette, J., Lobl, T., Greenberg, B., 1988. Amyloid precursor protein in senile plaques of Alzheimer disease. Lancet ii, 746.

Perry, G., Cras, P., Kawai, M., Mulvihill, P., Tabaton, M., 1991. Transformation of neurofibrillary tangles in the extracellular space. Bull. Clin. Neurosci. 56, 107–119.

Perry, G., Smith, M.A., 1993. Senile plaques and neurofibrillary tangles: what role do they play in Alzheimer's disease? Clin. Neurosci. 1, 199–203.

Perry, G., Smith, M.A., 1998. The case of vascular abnormalities in Alzheimer disease. Invest. Drugs Res. Alert (in press).

Praprotnik, D., Smith, M.A., Richey, P.L., Vinters, H.V., Perry, G., 1996a. Plasma membrane fragility in dystrophic neurites in senile plaques of Alzheimer's disease: an index of oxidative stress. Acta Neuropathol. 91, 1–5.

Praprotnik, D., Smith, M.A., Richey, P.L., Vinters, H.V., Perry, G., 1996b. Filament heterogeneity within the dystrophic neurites of senile plaques suggests blockage of fast axonal transport in Alzheimer's disease. Acta Neuropathol. 91, 226–235.

Richey, P.L., Siedlak, S.L., Smith, M.A., Perry, G., 1995. Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer disease: implications for disease pathogenesis. Biochem. Biophys. Res. Commun. 208, 657–663.

Rifenburg, R.P., Perry, G., 1995. Dystrophic neurites define diffuse as well as core-containing senile plaques in Alzheimer's disease. Neurodegeneration 4, 235–237.

Roberts, G.W., Gentleman, S.M., Lynch, A., Graham, D.I., 1991. BetaA4 amyloid protein deposition in brain after head trauma. Lancet 338, 1422–1423.

Roses, A.D., 1994. Apolipoprotein E affects the rate of Alzheimer's disease expression: β-amyloid burden is a secondary consequence dependent on ApoE genotype and duration of disease. J. Neuropathol. Exp. Neurol. 53, 429–437.

Roth, M., Tomlinson, B.E., Blessed, G., 1966. Correlation between scores for dementia and counts of senile plaques in cerebral grey matter of elderly subjects. Nature 209, 109–110.

Roth, M., Tomlinson, B.E., Blessed, G., 1967. The relationship between quantitative measures of dementia and of degenerative changes in the cerebral grey matter of elderly subjects. Proc. Royal Soc. Med. 60, 254–260.

Roth, G.S., Joseph, J.A., Mason, R.P., 1995. Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging. Trends Neurosci. 18, 203–206.

Saitoh, T., Masliah, E., Jin, L.W., Cole, G.M., Wieloch, T., Shapiro, I.P., 1991. Protein kinases and phosphorylation in neurologic disorders and cell death. Lab. Invest. 64, 596–616.

Sayre, L.M., Zagorski, M.G., Surewicz, W.K., Krafft, G.A., Perry, G., 1997. Mechanisms of neurotoxicity associated with amyloid β deposition and the role of free radicals in the pathogenesis of Alzheimer's disease: a critical appraisal. Chem. Res. Toxicol. 10, 518–526.

Selkoe, D.J., 1997. Alzheimer's disease: genotypes phenotypes and treatments. Science 275, 630–631.

Scheff, S.W., DeKosky, S.T., Price, D.A., 1990. Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol. Aging 11, 29–37.

Smith, C.D., Carney, J.M., Starke-Reed, P.E., Oliver, C.N., Stadtman, E.R., Floyd, R.A., Marksberry, W.R., 1991. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc. Natl. Acad. Sci. USA 88, 10540–10543.

Smith, M.A., 1998. Alzheimer disease. In: Bradley, R.J., Harris, R.A. (Eds.), International Review of Neurobiology, 42. Academic Press, San Diego, pp. 1–54.

Smith, M. A., Perry, G., 1997. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J. Neuropathol. Exp. Neurol. 56, 217.

Smith, M.A., Siedlak, S.L., Richey, P.L., et al., 1995a. Tau protein directly interacts with the amyloid β-protein precursor: Implications for Alzheimer's disease. Nature Med. 1, 365–369.

Smith, M.A., Sayre, L.M., Monnier, V.M., Perry, G., 1995b. Radical AGEing in Alzheimer's disease. Trends Neurosci. 18, 172–176.

Smith, M.A., Sayre, L.M., Perry, G., 1996a. Diabetes mellitus and Alzheimer's disease: glycation as a biochemical link. Diabetologia 39, 247.

Smith, M.A., Siedlak, S.L., Richey, P.L., Nagaraj, R.H., Elhammer, A., Perry, G., 1996b. Quantitative solubilization and analysis of insoluble paired helical filaments from Alzheimer disease. Brain Res. 717, 99–108.

Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M.F., Kowall, N., 1996c. Oxidative damage in Alzheimer's. Nature 382, 120–121.

Smith, M.A., Petot, G.J., Perry, G., 1997a. Diet and oxidative stress: a novel synthesis of epidemiological data on Alzheimer's disease. Alz. Dis. Rev. 2, 58–60.

Smith, M.A., Harris, P.L.R., Sayre, L.M., Beckman, J.S., Perry, G., 1997b. Widespread peroxynitrite-mediated damage in Alzheimer's disease. J. Neurosci. 17, 2653–2657.

Smith, M.A., Harris, P.L.R., Sayre, L.M., Perry, G., 1997c. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc. Natl. Acad. Sci. USA 94, 9866–9868.

Smith, M.A., Hirai, K., Hsiao, K., et al., 1998a. Amyloid-β deposition in Alzheimer transgenic mice initiates oxidative stress. J. Neurochem. 70, 2212–2215.

Smith, M.A., Wehr, K., Harris, P.L.R., Siedlak, S.L., Connor, J.R., Perry, G., 1998b. Abnormal localization of iron regulatory protein (IRP) in Alzheimer disease. Brain Res. 788, 232–236.

Snow, A.D., Mar, H., Nochlin, D., Sekiguchi, R.T., Kimata, K., Koike, Y., Wight, T.N., 1990. Early accumulation of heparan sulfate in neurons and in the β-amyloid protein-containing lesions of Alzheimer's disease and Down's syndrome. Am. J. Pathol. 137, 1253–1270.

Snowdon, D.A., Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A., Markesbery, W.R., 1997. Brain infarction and the clinical expression of Alzheimer disease: the Nun study. J. Am Med Assoc. 277, 813–817.

Sternberger, N.H., Sternberger, L.A., Ulrich, J., 1985. Aberrant neurofilament phosphorylation in Alzheimer disease. Proc. Natl. Acad. Sci. USA 82, 4274–4276.

Strittmatter, W.J., Weisgraber, K.H., Huang, D.Y., et al., 1993. Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc. Natl. Acad. Sci. USA 90, 8098–8102.

Suzuki, K., Terry, R.D., 1967. Fine structural localization of acid phosphatase in senile plaques in Alzheimer's presenile dementia. Acta Neuropathol. 8, 276–284.

Teller, J.K., Russo, C., DeBusk, L.M., et al., 1996. Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. Nature Med. 2, 93–95.

Terry, R.D., 1996. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J. Neuropath. Exp. Neurol. 55, 1023–1025.

Terry, R.D., Peña, C., 1965. Experimental production of neurofibrillary degeneration. J. Neuropathol. Exp. Neurol. 24, 200–210.

Tomlinson, B.E., Blessed, G., Roth, M., 1970. Observations on the brains of demented old people. J. Neurol. Sci. 11, 205–242.

Trojanowski, J.Q., Schmidt, M.L., Shin, R.-W., Bramblett, G.T., Goedert, M., Lee, V.M.-Y., 1993. PHFτ (A68): from pathological marker to potential mediator of neuronal dysfunction and degeneration in Alzheimer's disease. Clin. Neurosci. 1, 184–191.

Troncoso, J.C., Sternberger, N.H., Sternberger, L.A., Hoffman, P.N., Price, D.L., 1986. Immunocytochemical studies of neurofilament antigens in the neurofibrillary pathology induced by aluminum. Brain Res. 364, 295–300.

Van Lookeren Campagne, M., Gill, R., 1998. Cell cycle-related gene expression in the adult rat brain: selective induction of cyclin G1 and $p21^{WAF1/CIP1}$ in neurons following focal cerebral ischemia. Neuroscience 84, 1097–1112.

Whitson, J.S., Mims, M.P., Strittmatter, W.J., Yamaki, T., Morrisett, J.D., Appel, S.H., 1994. Attenuation of the neurotoxic effect of Aβ amyloid peptide by apolipoprotein E. Biochem. Biophys. Res. Commun. 199, 163–170.

Wilcock, G.K., Esiri, M.M., 1982. Plaques, tangles and dementia: a quantitative study. J. Neurol. Sci. 56, 343–356.

EXHIBIT D

Volume 46(6): 731–735, 1998
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

ARTICLE

Cytochemical Demonstration of Oxidative Damage in Alzheimer Disease by Immunochemical Enhancement of the Carbonyl Reaction with 2,4-Dinitrophenylhydrazine

Mark A. Smith, Lawrence M. Sayre, Vernon E. Anderson, Peggy L.R. Harris, M. Flint Beal, Neil Kowall, and George Perry

Institute of Pathology (MAS,PLRH,GP), Department of Chemistry (LMS), and Department of Biochemistry (VEA), Case Western Reserve University, Cleveland, Ohio; Department of Neurology (MFB), Harvard Medical School, Boston, Massachusetts; and Department of Veterans Affairs (NK), Bedford, Massachusetts

SUMMARY Formation of carbonyls derived from lipids, proteins, carbohydrates, and nucleic acids is common during oxidative stress. For example, metal-catalyzed, "site-specific" oxidation of several amino acid side-chains produces aldehydes or ketones, and peroxidation of lipids generates reactive aldehydes such as malondialdehyde and hydroxynonenal. Here, using in situ 2,4-dinitrophenylhydrazine labeling linked to an antibody system, we describe a highly sensitive and specific cytochemical technique to specifically localize biomacromolecule-bound carbonyl reactivity. When this technique was applied to tissues from cases of Alzheimer disease, in which oxidative events including lipoperoxidative, glycoxidative, and other oxidative protein modifications have been reported, we detected free carbonyls not only in the disease-related intraneuronal lesions but also in other neurons. In marked contrast, free carbonyls were not found in neurons or glia in age-matched control cases. Importantly, this assay was highly specific for detecting disease-related oxidative damage because the site of oxidative damage can be assessed in the midst of concurrent age-related increases in free carbonyls in vascular basement membrane that would contaminate biochemical samples subjected to bulk analysis. These findings demonstrate that oxidative imbalance and stress are key elements in the pathogenesis of Alzheimer disease.
(J Histochem Cytochem 46:731–735, 1998)

KEY WORDS
Alzheimer disease
carbonyls
2,4-dinitrophenylhydrazine
oxidative damage

ALTHOUGH OXIDATIVE STRESS and consequent free radical damage are involved as a major cytopathological mechanism in a number of diseases and in normal aging, methods to document free radical involvement have not grown at the same rate as hypotheses. This gap has stemmed primarily from problems in quantifying free radicals in vivo, because they are rapidly removed by antioxidants or quenched by reaction with biopolymers. In addition, normal metabolism generates abundant free radicals, such that detecting them without knowing the balance of oxidants and antioxidants is not biologically meaningful. This aspect has been circumvented by methods to detect excess radical production. Although detection can be performed using exogenous agents that are specifically modified by free radicals, such as spin traps (Young et al. 1996), endogenous macromolecules can also be used as indicators because protein, DNA, and lipids are all chemically changed by oxidative stress (Stadtman 1993).

Modifications of proteins and polynucleotides take two forms: (a) adduction reactions by highly reactive intermediate products of lipid peroxidation or glycation, and (b) direct oxidative modification of the macromolecules. In the case of proteins, powerful oxidizing agents such as the hydroxyl radical directly modify amino acid side-chains, resulting in a diverse array of altered amino acids that are used to assess oxidative damage (Stadtman 1993). Among the most widespread of these modifications, and one that is considered specific for oxidative damage, is the generation of free carbonyls that are not present on nonoxidized pro-

Correspondence to: Mark A. Smith, Inst. of Pathology, Case Western Reserve U., 2085 Adelbert Road, Cleveland, OH 44106.
Received for publication June 4, 1997; accepted January 7, 1998 (7A4359).

 0022-1554/98/$3.30

teins (Levine et al. 1994). 2,4-Dinitrophenylhydrazine (DNP-H) reacts with free carbonyls and can therefore serve as a marker for the extent of oxidative damage to a given protein (Szweda et al. 1993). However, although this method has been validated as an indicator of oxidative damage for a number of tissues and isolated proteins (Smith et al. 1991), the procedure does not provide histological resolution. Such cellular localization is essential for analysis of tissue when one considers that the vascular basement membrane and extracellular matrix proteins, which can contaminate many biochemical samples, contain abundant oxidative modifications as a consequence of their long half-lives.

Here we report a highly specific and sensitive method to cytologically define oxidative damage that is based on the DNP-H reaction with free carbonyls but involves subsequent immunocytochemical enhancement. The technique is demonstrated using tissue sections from cases of Alzheimer disease and controls, because glycoxidative and lipoperoxidative damage are well documented in the pathological lesions of Alzheimer disease, neurofibrillary tangles and senile plaques (Yan et al. 1994; Smith et al. 1994,1995a,b,1996,1997; Montine et al. 1996; Sayre et al. 1997).

Materials and Methods

Tissue

The hippocampus extending from the entorhinal cortex to the full hippocampus from six cases of Alzheimer disease (ages 76–90), three age-matched controls (ages 70–78), and three younger controls (ages 32–64) was fixed in Methacarn (methanol:chloroform:acetic acid 60:30:10) at 4C for 24 hr. Tissue was dehydrated through ascending ethanol solutions and xylene and then embedded in paraffin (~60C). Sections of 6 μm sections were prepared with a microtome (Leica), placed on Silane-coated glass slides (Sigma; St Louis, MO), and sequentially deparaffinized in xylene and rehydrated in descending ethanol. Endogenous peroxidase activity was inactivated by treating with 3% H_2O_2 in methanol for 20 min to block artifactual staining from endogenous peroxidase activity (Sternberger 1986). Although H_2O_2 might itself, or synergistically with tissue-bound iron, create carbonyl residues, deletion of this step had no effect on subsequent DNP-H labeling for the experiments shown here. However, a control that omits the H_2O_2 treatment step should be performed with each experimental paradigm. After blocking endogenous peroxidases, tissue was hydrated in ethanol (70, 50, and 30%) to 50 mM Tris-HCl, 0.15 M NaCl, pH 7.6 (TBS).

Dinitrophenyl Labeling

Sections were covered with 0.1–0.001% DNP-H in 2 N HCl. All incubations were done in a humidified plastic box. After a 1-hr incubation at room temperature (RT), sections were exhaustively rinsed in TBS, followed by a 30-min incubation in 10% normal goat serum (NGS) to block nonspecific binding sites. After rinsing with 1% NGS/TBS, a rat monoclonal antibody (LO-DNP-2; Zymed, San Francisco, CA) to dinitrophenyl (DNP) was diluted 1:100 in 1% NGS/TBS and incubated with the sections at 4C for 16 hr. Sections were then rinsed with 1% NGS/TBS followed by goat antiserum to rat IgG (Boehringer–Mannheim; Indianapolis, IN) diluted 1:50 with 1% NGS/TBS. After rinsing in 1% NGS/TBS, rat peroxidase–anti-peroxidase complex (ICN) (1:250) in the same buffer was incubated with the section at RT for 1 hr, after which it was rinsed with 1% NGS/TBS. Peroxidase activity was localized by development for 5–10 min with 0.015% H_2O_2 in 50 mM Tris-HCl, pH 7.6, with 0.75 mg/ml 3,3′-diaminobenzidine (Sigma). Development of the sections was directly monitored for maximal contrast under the ×10 objective of a Zeiss Axioskop 20 microscope.

Chemical and immunochemical controls were used to define carbonyl-specific binding. Chemical reduction of free carbonyls and Schiff bases was performed by incubating sections with 25 mM sodium borohydride ($NaBH_4$) in 80% methanol for 30 min at RT before incubation with DNP-H. Specific reduction of Schiff bases, while leaving carbonyls intact, was performed with 50 mM sodium cyanoborohydride in 0.1 M phosphate buffer, pH 6.0, for 1 hr at RT. Immunochemical specificity was demonstrated by omission of the antibody to DNP or the DNP-H treatment. Immunoabsorption of the antibody to DNP was performed by incubating the antibody (1:100) with 5 mM pyruvate 2,4-dinitrophenylhydrazone (stock 1 mg/ml in ethanol) at 4C for 16 hr and comparing the resulting immunoreactivity with unabsorbed antibody that had been similarly treated with 5% ethanol.

Results

DNP-H derivatizes free carbonyls on biomacromolecules, leading to DNP adduction to the carbonyl-containing biomacromolecule. In contrast, reaction of DNP-H with Schiff bases formed between exogenous carbonyl compounds and proteinaceous lysine side-chains leads to release of soluble dinitrophenylhydrazones with no fixation of the DNP moiety to the tissue. By examining the sites of DNP adduction from reaction with DNP-H, localization of oxidative damage at the cytological level can be achieved. Without subsequent antibody enhancement, DNP-H showed only faint yellow staining of the large blood vessels in the brains of aged individuals. No staining of other brain cells or of the pathological changes of Alzheimer disease was seen (data not shown). DNP-H reaction and consequent DNP adduction to the walls of large vessels may not be surprising because extracellular matrix proteins abundant in vessels accumulate oxidative modifications, probably owing to their long half-life (Salomon et al. 1997). Consistent with this interpretation, the large vessels of the aged individuals stained more intensely than those of the young (data not shown) (Salomon et al 1997). Although this assay was specific for free carbonyls, e.g., it is blocked with prior reduction of carbonyls with $NaBH_4$, but not $NaCNBH_3$, the value of the direct detection assay with DNP-H is limited because it does not detect the pathological sites of

known oxidative damage in the lesions of Alzheimer disease. Therefore, sensitivity was increased by coupling the chemical reaction of DNP-H to an enzyme-linked immunocytochemical technique to detect bound DNP. This method showed prominent DNP adduct formation not only in large vessels (not shown) but also in the cell bodies and apical dendrites of the pyramidal neurons of the hippocampus in cases of Alzheimer disease (Figures 1A and 1C). In contrast, DNP adducts were undetectable in the neuronal cytoplasm of young or age-matched controls and in microvessels (Figure 1B). In addition to cytoplasmic staining of populations of large pyramidal neurons, intraneuronal neurofibrillary tangles were also recognized (Figure 1A, arrows). Other populations of neurons, e.g., dentate granule cells, were also stained, but with lesser intensity. Senile plaques (including dystrophic neurites, β-amyloid deposits, microglia, astrocytes, and adjacent oligodendrocytes) showed no reaction with DNP-H.

The specificity of the method to detect free carbonyls was demonstrated by chemical and immunochemical controls. In the former case, reduction of free carbonyls with $NaBH_4$ blocked DNP-H binding (Figure 1D), whereas reduction of Schiff bases with $NaCNBH_3$ was again without effect (not shown). Immunochemical validation was demonstrated by performing all of the following: omitting DNP-H treatment, omitting the antibody to DNP, or absorbing the antibody to DNP with the DNP-H derivative of pyruvate (Figure 1E). These immunochemical controls were critical to ensure the use of an antibody detection technique that is highly sensitive and specific, e.g., we found several different rabbit antisera to DNP that showed such high background staining for neurons that it was often difficult to note specific staining, whereas the rat monoclonal antibody showed no background staining.

Tissue was fixed in the non-crosslinking fixative Methacarn (methanol:chloroform:acetic acid 60:30:10), but we had similar results using unfixed or samples fixed in 80% ethanol. In contrast, tissue fixed in formaldehyde-based fixatives, as well as sections from tissue fixed in Methacarn but later treated with formalde-

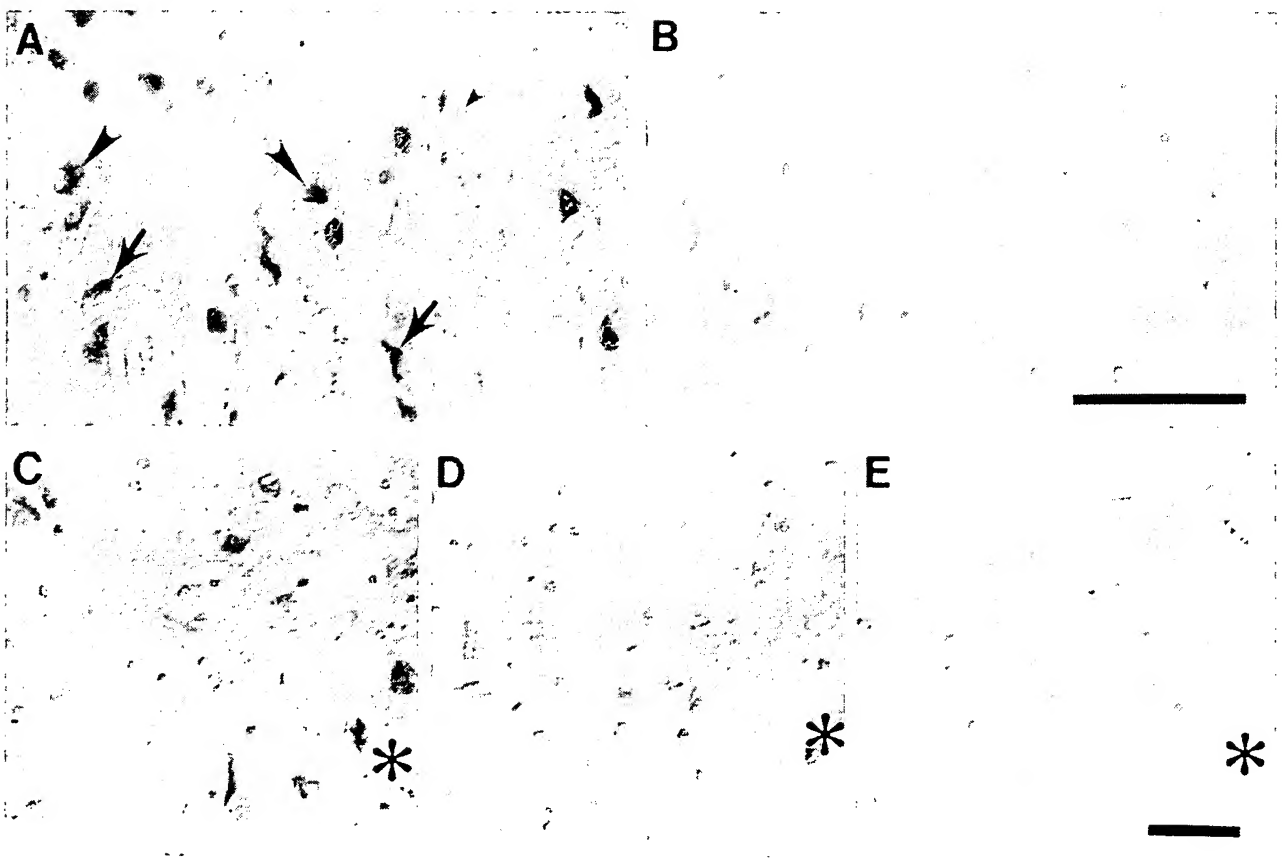


Figure 1 Free carbonyls were detected in cases of Alzheimer disease (A) not only in the pathological lesions of the disease, i.e., neurofibrillary tangles (arrows) but also in cytoplasm of neurons (large arrowheads). By contrast, in age-matched (B) and young controls none are labeled. DNP-H adduction in a case of Alzheimer disease (C) is completely blocked by prior reduction by $NaBH_4$ (D), omission of DNP-H, or adsorption of anti-DNP with DNP-pyruvate (E). * indicates landmark present in each adjacent serial section (C–E). Bars = 50 μm.

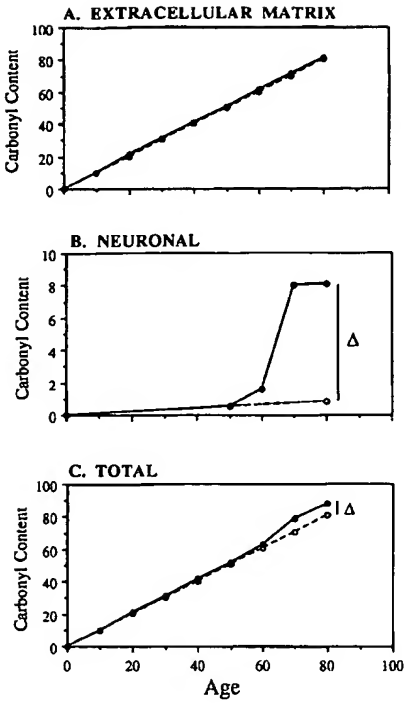


Figure 2 Schematic representation of the relationship between aging- (·····) and disease (———)-related oxidative damage, showing that the accumulation of damage in long-lived extracellular matrix proteins (**A**) represents the vast majority of total modifications in tissue (**C**). Therefore, using whole-tissue homogenates, disease-related increases (Δ) in neuronal carbonyls (**B**) might not be appreciated in relation to the total (**C**). Using in situ techniques as described herein, the increased neuronal carbonyls are readily apparent. Note: Scale bars for carbonyls in **A** and **C** are a magnitude higher than in **B**.

hyde, displayed nonspecific DNP-H adduction throughout the section, making them unsuitable for study.

Discussion

2,4-Dinitrophenylhydrazine (DNP-H), when coupled to immunochemical enhancement, can detect oxidative damage produced by a pathological condition, Alzheimer disease. It is of interest that the adduction of DNP-H was limited to cell bodies of neurons. This is essentially the same localization noted for adducts of the highly reactive lipid peroxidation product hydroxynonenal (Sayre et al. 1997) and for nitrotyrosine (Smith et al. 1997), the result of peroxynitrite-mediated damage. In all these cases, neuronal cytoplasm showed evidence of damage whereas glia and senile plaques showed none.

Absence of oxidative damage in the senile plaque is surprising in light of reports that senile plaques are the source of oxygen radicals in Alzheimer disease. First, β-amyloid, the primary component of senile plaques, is suggested to produce free radicals through a novel mechanism (Butterfield et al. 1994). Second, β-amyloid interaction with the RAGE receptor of microglia leads to free radical production (Yan et al. 1996). Third, senile plaques accumulate iron, which may be available to generate hydroxyl radicals through Fenton chemistry (Grundke-Iqbal et al. 1990; Connor et al. 1992; Levine et al. 1994; Jefferies et al. 1996; Smith et al. 1997). Yet, when we examine free carbonyls, as here, lipid peroxide adducts (Sayre et al. 1997), or peroxynitrite-mediated damage (Smith et al. 1997), we find no increase in damage in senile plaques. Absence of oxidative damage in senile plaques argues either that β-amyloid and the surrounding cells are not susceptible to oxidative damage, that radical production by senile plaques is balanced by antioxidant defenses, or that some neuron-specific macromolecules are very susceptible to oxidation. Although it can be argued that β-amyloid, because of its tight β-structure and low lysine content, may be less susceptible than lysine-rich cytoskeletal proteins, cytoskeletal proteins also accumulate as paired helical filaments in the abnormal neurites surrounding senile plaques. Why cytoskeletal proteins exhibit no oxidative damage in senile plaque neurites and do exhibit damage while in the neuronal cell body as neurofibrillary tangles is a major unresolved issue of our findings. Nevertheless, our results do not support the senile plaque as the primary site of free radical imbalance. Because reactive oxygen species (ROS) have short diffusion distances through tissue, our findings instead implicate an ROS source within the cell body. Although the paired helical filaments might play this role (Yan et al. 1994; Smith et al. 1995a), their absence in most neurons showing increased free carbonyls, as well as their presence in senile plaque neurites, does not strongly argue for their involvement in neuronal oxidative damage. Another possibility is mitochondria, because mitochondrial respiration is associated with ROS production and because significant mitochondrial abnormalities (Davis et al. 1997) and altered metabolism (Smith et al. 1997) are found in AD. In this regard, the specificity of damage to neuronal vs glial cytoplasm is expected if ROS depends on high metabolic activity, because neurons are among the most oxidative cells of the body. This, together with possible differences in either the susceptibility or the clearance of damaged proteins, may be responsible for the observed difference. It is tempting to speculate that neuronal damage involves not only the cytoskeletal proteins that bear glycoxidative (Ledesma et al. 1994; Yan et al. 1995) and lipoxidative (Smith et al., unpublished observation) adducts but also nuclear components. Furthermore, the latter finding is consistent with the increased DNA fragmentation noted in Alzheimer disease (Su et al. 1994), because

oxidative strand cleavage and repair of oxidative base modification are major causes of DNA fragmentation that may be incorrectly ascribed to apoptosis (Tsang et al. 1996).

Our finding of increased neuronal DNP-H adduction is consistent with biochemical studies demonstrating increased DNP-H-derived adducts by assay of tissue homogenates from normal aging and Alzheimer disease (Smith et al. 1991). However, in light of the large number of free carbonyls in the vasculature of all samples, particularly of the aged, the specificity and sensitivity of whole-tissue analysis to detect disease related increases, particularly for an age-related disease, are limited. Therefore, disease-related neuronal damage could be masked by damage to vessels when whole-tissue homogenates are used (Figure 2).

Another approach is to use the enhanced DNP-H immunochemical technique to identify specific proteins modified on immunoblots or by isolation (Smith et al. 1991; Shacter et al. 1994; Nakamura and Goto 1995). Coupling of cytological and biochemical approaches not only may give greater specificity and sensitivity to oxidative damage measurement but may also lead to a greater appreciation of its dynamic nature.

Acknowledgments

Supported by grants from the National Institutes of Health (AG09287, AG13846, and AG14249), by the Alzheimer's Association, and by the American Health Assistance Foundation.

Literature Cited

Butterfield DA, Hensley K, Harris M, Mattson M, Carney J (1994) β-amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. Biochem Biophys Res Commun 200:710–715

Connor JR, Menzies SL, St. Martin SM, Mufson EJ (1992) A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. J Neurosci Res 31:75–83

Davis RE, Miller S, Herrnstadt C, Ghosh SS, Fahy E, Shinobu LA, Galasko D, Thal LJ, Beal MF, Howell N, Parker WD Jr. (1997) Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc Natl Acad Sci USA 94:4526–4531

Grundke-Iqbal I, Fleming J, Tung YC, Lassmann H, Iqbal K, Joshi JG (1990) Ferritin is a component of the neuritic (senile) plaque in Alzheimer disease. Acta Neuropathol (Berl) 81:105–110

Jefferies WA, Food MR, Gabathuler R, Rothenberger S, Yamada T, Yasuhara O, McGeer PL (1996) Reactive microglia specifically associated with amyloid plaques in Alzheimer's disease brain tissue express melanotransferrin. Brain Res 712:122–126

Ledesma MD, Bonay P, Colaco C, Avila J (1994) Analysis of microtubule-associated protein tau glycation in paired helical filaments. J Biol Chem 269:21614–21619

Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

Montine TJ, Huang DY, Valentine WM, Amarnath V, Saunders A, Weisgraber KH, Graham DG, Strittmatter WJ (1996) Crosslinking of apolipoprotein E by products of lipid peroxidation. J Neuropathol Exp Neurol 55:202–210

Nakamura A, Goto S (1995) Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. J Biochem 119:768–774

Salomon RG, Subbanagounder G, ONeil J, Kaur K, Smith MA, Hoff HF, Perry G, Monnier VM (1997) Levuglandin E_2-protein adducts in human plasma and vasculature. Chem Res Toxicol 10:536–545

Sayre LM, Zelasko DA, Harris PLR, Perry G, Salomon RG, Smith MA (1997) 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. J Neurochem 68:2092–2097

Shacter E, Williams JA, Lim M, Levine RL (1994) Differential susceptibility of plasma proteins to oxidative modification: examination by western blot immunoassay. Free Radic Biol Med 17:429–437

Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Marksberry WR (1991) Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 88:10540–10543

Smith MA, Harris PLR, Sayre LM, Beckman JS, Perry G (1997) Widespread peroxynitrite-mediated damage in Alzheimer's disease. J Neurosci 17:2653–2657

Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N (1996) Oxidative damage in Alzheimer's. Nature 382:120–121

Smith MA, Rudnicka-Nawrot M, Richey PL, Praprotnik D, Mulvihill P, Miller CA, Sayre LM, Perry G (1995b) Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease. J Neurochem 64:2660–2666

Smith MA, Sayre LM, Monnier VM, Perry G (1995a) Radical AGEing in Alzheimer's disease. Trends Neurosci 18:172–176

Smith MA, Taneda S, Richey PL, Miyata S, Yan S-D, Stern D, Sayre LM, Monnier VM, Perry G (1994) Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proc Natl Acad Sci USA 91:5710–5714

Stadtman ER (1993) Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu Rev Biochem 62:797–821

Sternberger LA (1986) Immunocytochemistry. New York, Wiley

Su JH, Anderson AJ, Cummings BJ, Cotman CW (1994) Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 5:2529–2533

Szweda LI, Uchida K, Tsai L, Stadtman ER (1993) Inactivation of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal. Selective modification of an active-site lysine. J Biol Chem 268: 3342–3347

Tsang SY, Tam SC, Bremner I, Burkitt MJ (1996) Copper-1,10-phenanthroline induces internucleosomal DNA fragmentation in HepG2 cells, resulting from direct oxidation by the hydroxyl radical. Biochem J 317:13–16

Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. Nature 382:685–691

Yan S-D, Chen X, Schmidt A-M, Brett J, Godman G, Zou Y-S, Scott CW, Caputo C, Frappier T, Smith MA, Perry G, Yen S-H, Stern D (1994) Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci USA 91:7787–7791

Yan SD, Yan SF, Chen X, Fu J, Chen M, Kuppusamy P, Smith MA, Perry G, Godman GC, Nawroth P, Zweier JL, Stern D (1995) Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid β-peptide. Nature Med 1:693–699

Young HK, Floyd RA, Maidt ML, Dynlacht JR (1996) Evaluation of nitrone spin-trapping agents as radioprotectors. Radiat Res 146:227–231

EXHIBIT E

ELSEVIER

Brain Research Reviews 30 (1999) 153–163

BRAIN RESEARCH REVIEWS

www.elsevier.com/locate/bres

Full-length review

Neurodegenerative disorders: the role of peroxynitrite

François Torreilles [a], Saïda Salman-Tabcheh [b,1], Marie-Christine Guérin [b], Jean Torreilles [b,*]

[a] *CNRS UMR 9921 UFR Pharmacie, 15 av. Charles Flahault, 34060 Montpellier Cedex 2, France*

[b] *IFR 56 "Eugène Bataillon", Université Montpellier II, cc 91, 2 place Eugène Bataillon, 34095 Montpellier Cedex 5, France*

Accepted 22 June 1999

Abstract

Inflammatory reaction is thought to be an important contributor to neuronal damage in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and the parkinsonism dementia complex of Guam. Among the toxic agents released in brain tissues by activated cells, we focus attention in this review on peroxynitrite, the product of the reaction between nitric oxide (NO) and superoxide. Peroxynitrite is a strong oxidizing and nitrating agent which can react with all classes of biomolecules. In the CNS it can be generated by microglial cells activated by pro-inflammatory cytokines or β-amyloid peptide (β-A) and by neurons in three different situations: hyperactivity of glutamate neurotransmission, mitochondrial dysfunction and depletion of L-arginine or tetrahydrobiopterin. The first two situations correspond to cellular responses to an initial neuronal injury and the peroxynitrite formed only exacerbates the inflammatory process, whereas in the third situation the peroxynitrite generated directly contributes to the initiation of the neurodegenerative process.

Keywords: Alzheimer's disease; Parkinson's disease; Multiple sclerosis; Amyotrophic lateral sclerosis; Peroxynitrite

Contents

1. Introduction 154

2. Formation of peroxynitrite and reactions with biological compounds 154

3. The formation of peroxynitrite in the CNS 155
   3.1. By reaction of NO from Type-I NOS with O_2^- from the mitochondrial respiratory chain 156
   3.2. By activation of microglial cells 156
   3.3. By impairment of oxidative metabolism 157
   3.4. By Type-I NOS activity in arginine- or tetrahydrobiopterin-depleted conditions 158

4. Relevance of peroxynitrite to neurodegenerative disorders 158
   4.1. Amyotrophic lateral sclerosis 158
   4.2. Multiple sclerosis 159
   4.3. Alzheimer's disease 159
   4.4. Parkinson's disease 159

5. Peroxynitrite as a mediator of neuronal damage 160

Acknowledgements 160

References 160

* Corresponding author. Fax: +33-4-6714-3429; E-mail: jtorreil@univ-montp2.fr

[1] Present address: Faculté des Sciences III — Tripoli nord-Liban BP: 826, Lebanon.

0165-0173/99/$ - see front matter
PII: S0165-0173(99)00014-4

1. Introduction

The association of many proteins known to be involved in inflammatory processes with senile plaques and microfibrillary tangles in chronic neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and the parkinsonism dementia complex of Guam is consistent with the autotoxic loop hypothesis developed by McGeer and McGeer [56,57] (Fig. 1).

These authors assume that in a first step, an initial insult promotes neuronal damage with the deposit of debris that activates the microglial cells leading to the release of cytotoxic agents and initiation of the classical complement cascade [80]. The toxic products thus produced cause neuronal death which in turn spurs an inflammatory reaction. This reaction can be a self-sustaining autodestructive force in which cell response injures bystander neurons and produces further lesions. Thus, a vicious cycle of damage can be generated and sustained.

Accumulating evidence has implicated not only reactive oxygen radicals but also nitric oxide (NO) in the inflammatory process. Here we provide a brief account of present knowledge regarding the product of the reaction between NO and superoxide (O_2^-), namely, peroxynitrite, which is thought to mediate the toxic action of these species [6] and play an important role in sustaining as well as initiating the inflammatory autotoxic loop in the pathogenesis of neurodegenerative disorders.

2. Formation of peroxynitrite and reactions with biological compounds

Radicals like NO and O_2^- have little propensity to react with non-radical biomolecules because such reactions are electronically unbalanced and do not result in additional bond formation which favors such reactions. However, the reaction rate for combination between NO and O_2^- occurs at the near diffusion-limited rate of 4.3 to 6.7×10^9 M^{-1} s^{-1} generating peroxynitrite [36,60,68], a potent oxidant and nitrating agent capable of attacking and modifying proteins, lipids and DNA as well as depleting antioxidant defences (Fig. 2). Because nitric oxide reacts with O_2^- three-fold faster than superoxide dismutase (SOD) ($k = 2.3 \times 10^9$ M^{-1} s^{-1}), NO is the only known biomolecule capable of out competing SOD for available O_2^-. Thus, NO and O_2^- can be considered as components of a binary chemical weapon which, when mixed, produce a toxic compound: peroxynitrite.

The peroxynitrite shows a high reactivity toward aqueous CO_2 leading to the formation of very reactive intermediates that are more efficient nitrating species than peroxynitrite itself [79]. CO_2 is ubiquitous in tissue and then the reaction of peroxynitrite with CO_2 accounts for a large fraction of the peroxynitrite reactivity in vivo. In CNS this reactivity could be a factor which exacerbates nitration of tyrosine residues particularly in pathologies which promote acidosis.

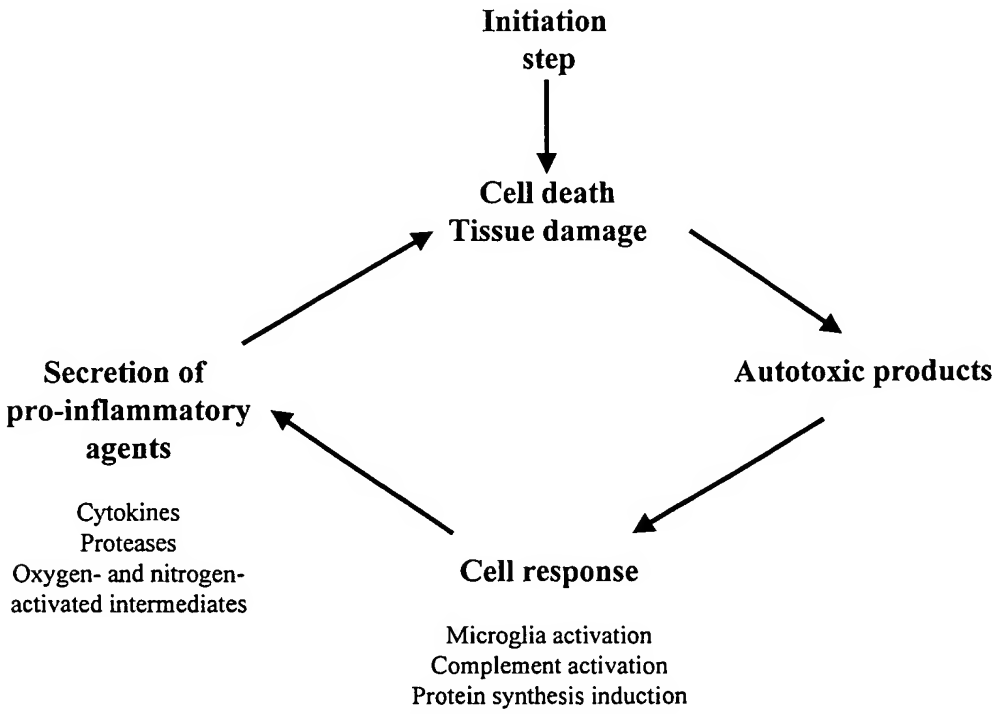


Fig. 1. The autotoxic loop model proposed for the inflammatory mechanism in neurodegenerative disorders.

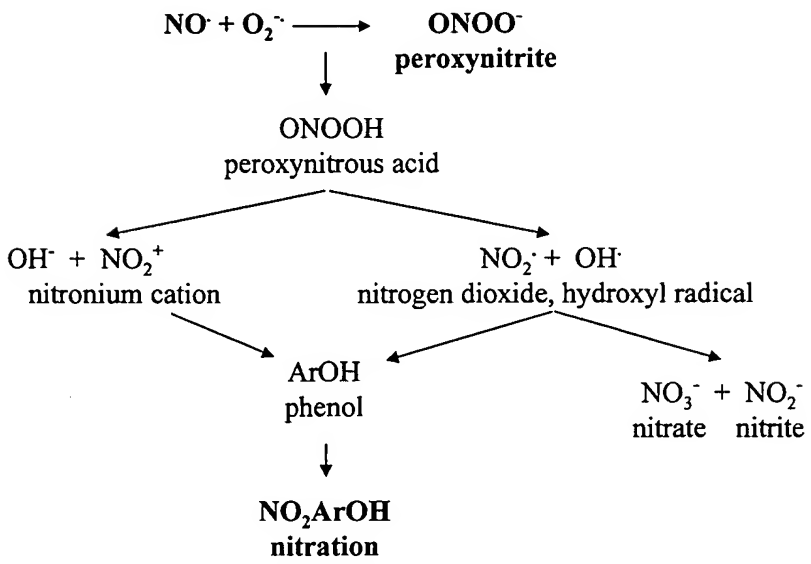


Fig. 2. Formation and chemical reactivity of peroxynitrite.

Peroxynitrite reacts in complex ways with different relevant biomolecules. It has been shown to induce lipid peroxidation, cause DNA strand scission, oxidize cysteine, lysine, methionine and histidine residues and nitrate heterocyclic compounds like tryptophan and guanine or phenolics like tyrosine [20,42,46]. This last reaction, leading to 3-nitro-L-tyrosine, may be injurious via several possible mechanisms: (i) alteration of tyrosine phosphorylation-dependent signaling because nitrotyrosine resembles phosphotyrosine and may irreversibly block tyrosine phosphorylation, (ii) modification of protein conformation and enhancement of proteolysis by introduction of a negative charge in hydrophobic tyrosine and (iii) initiation of autoimmune processes because nitrophenols are known to be highly antigenic [6].

3. The formation of peroxynitrite in the CNS

Because the concentrations of SOD and O_2^- in a given tissue are relatively constant, the primary driving force for peroxynitrite formation is the NO concentration. In the CNS, three NO-synthase isoforms, neuronal Type-I NOS, inducible Type-II NOS and endothelial Type-III NOS, can generate NO. They catalyze the formation of NO and citrulline from L-arginine via the intermediate N_ω-hydroxy-

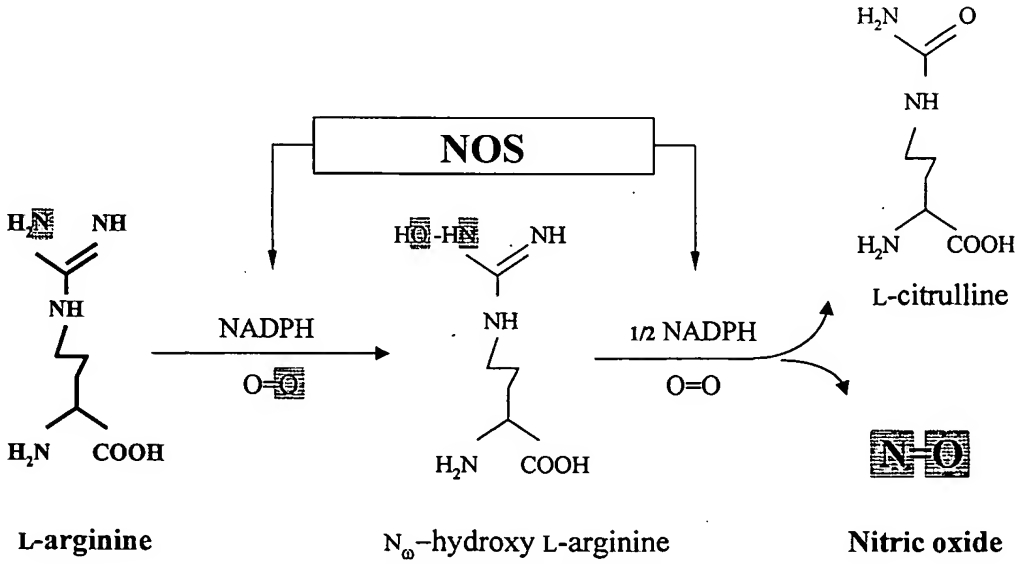


Fig. 3. Schematic representation of NOS-catalyzed reactions.

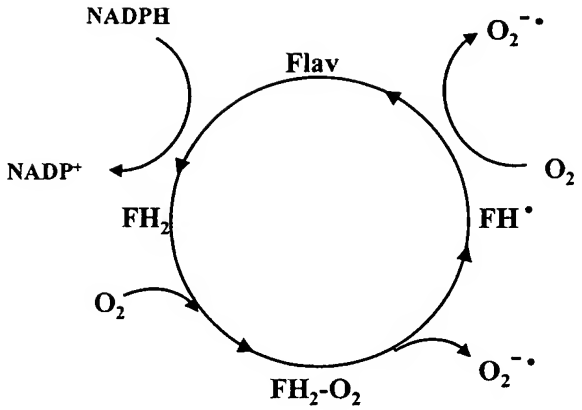


Fig. 4. Production of O_2^- by autoxidation of flavoproteins.

arginine (Fig. 3). (This review will not include a great deal of detail about the biochemistry and pharmacology of NOSs as there are many excellent review articles [53,78,80]).

The citrulline formed can leave the cell or be converted back to arginine via the enzymes argininosuccinate lyase located in Bergmann glia and argininosuccinate synthetase located in basket cells, stellate cells, Golgi cells and mossy fibers. Type-I NOS has been detected in the cerebellum, the hypothalamus, the striatum, the hippocampus and the medulla oblongata. Type-II NOS is located predominantly in microglia and astrocytes [43]. Type-III NOS has been detected in microvessels and motor neurons from rodents [11,21,24,25,41,64,66] and humans [2,13,14].

O_2^- can be formed as a by-product of the mitochondrial respiratory chain generated primarily by autoxidation of flavoproteins [78] (Fig. 4) or during the respiratory burst of phagocytes via the activation of NADPH-oxidase [48]. Consequently, peroxynitrite can be generated in the CNS in different ways.

3.1. By reaction of NO from Type-I NOS with O_2^- from the mitochondrial respiratory chain

In normal brain, such a pathway seems to be unlikely since Okabe et al. [65] recently shown that SOD, the enzyme which scavengers O_2^-, colocalizes with NOS in the hippocampus and the cerebellum where NO plays an important role as neuromediator. However, in the case of hyperactivity of glutamate neurotransmission [61] and mitochondrial dysfunction, including focal trauma, epileptic seizure, ALS, PD and other neurodegenerative diseases, the excessive intracellular calcium accumulation leads to an abnormal activation of Ca^{2+}-dependent enzymes, including the Type-I NOS. Then, the excess NO may react with O_2^- to form peroxynitrite [10,72,74,85].

Moreover, excess NO may also promote an increase of O_2^- production by binding to the heme moiety of the cytochrome *c* oxidase, the complex IV of the electron transport chain in the mitochondrial membrane. This binding may cause a transient inhibition of the electron flow yielding an increase in O_2^- synthesis by complexes I and III (which are relatively insensitive to NO) thus favoring the intracellular production of peroxynitrite [12] (Fig. 5).

3.2. By activation of microglial cells

Microglial cells account for approximately 20% of the total glial population in the CNS. They belong to the mononuclear phagocyte system and form the resident macrophages in the brain, the spinal cord and the retina [47].

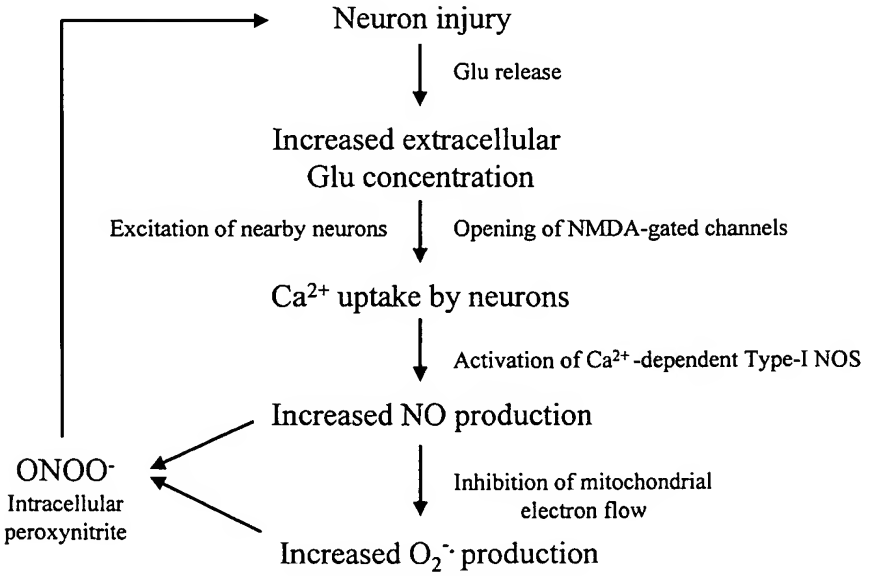


Fig. 5. Relationship between the hyperactivity of glutamate (Glu) neurotransmission and peroxynitrite formation.

Microglial cells are normally in a resting and immunodepressed state, but in most inflammatory CNS disorders, they become gradually activated. Fully activated microglial cells have a strong respiratory burst activity and generate O_2^-. As other phagocytes, they also produce growth factors [16] to promote tissue repair and cytokines, proteases, Type-II NO synthase and prostanoids to destroy invading micro-organisms and remove potentially deleterious debris. In vitro experiments have shown that activated microglial cells can become neurotoxic by producing peroxynitrite from NO and O_2^- [11,37].

Microglial cells can be activated by pro-inflammatory cytokines, IL-1, IL-6 and TNFα, as well as by β-amyloid peptide (β-A) [82,90], the first 42 amino acids of amyloid precursor protein (APP) (Fig. 6). Cleavage of this protein, which is composed of about 700 amino acids, generates soluble cleavage peptides, including β-A. The soluble β-A monomer has strong propensity for self-aggregation into an insoluble aggregated β-pleated sheet form [45,55]. Nilson et al. [63] described three states of β-A able to promote the simultaneous production of O_2^- and NO, and thus, peroxynitrite: the soluble β-A monomer, a diffuse amyloid deposit and mature filamentous amyloid plaques. The last two forms are more potent in activating microglia.

The β-A soluble form is present in the cortical extracellular space and in the cerebrospinal fluid. It can initiate microglial cell activation, NO production, inflammation and necrotic cell death by disturbing calcium ion gradient across the cell [4,35].

The insoluble form of β-A abnormally accumulates in the brain and degenerative neurons in many neurodegenerative diseases. It has been shown by Le et al. [49,50] to cause apoptotic cell death in vitro through the generation of NO. Recent evidence indicates that lipoproteins transport the hydrophobic β-A in cerebrospinal fluid. Lipoproteins favor microglial cell activation and β-A endocytosis [9,87].

The greater part of data on microglia NO generation were obtained using rodent cells, whereas Colton et al. [15] by Greiss reaction on microglia cultures, Walker et al. [89] by measuring Type-I NOS gene expression on human microglia cultures, and Vodovotz et al. [88] by immunocytochemistry on brains from normal subjects and Alzheimer patients have shown that, mouse and rat microglia effectively secrete large amounts of NO while human and hamster microglia generate very small even no amounts of NO. As for human monocytes, the NO production from human microglia remains to be formally proved. But, in vivo, this demonstration is made difficult because astrocytes and microglia function cooperatively in generating the innate immune response of brain [57].

3.3. By impairment of oxidative metabolism

A reduction in thiamine-dependent enzymes, leading to cell impairment has been observed in many neurodegenerative diseases [54,75]. Calingasan et al. [11] described a rodent thiamine deficiency model to study the mechanisms involved in neuronal damage. These authors observed a breakdown of the blood–brain barrier associated with enhanced expression of Type-III NOS in microvessels and

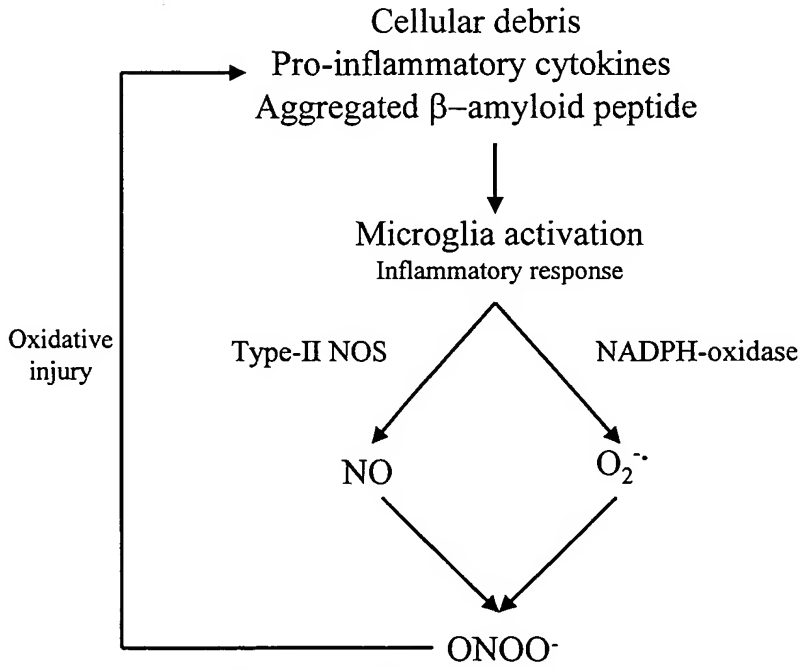


Fig. 6. Production of peroxynitrite by activated microglial cells.

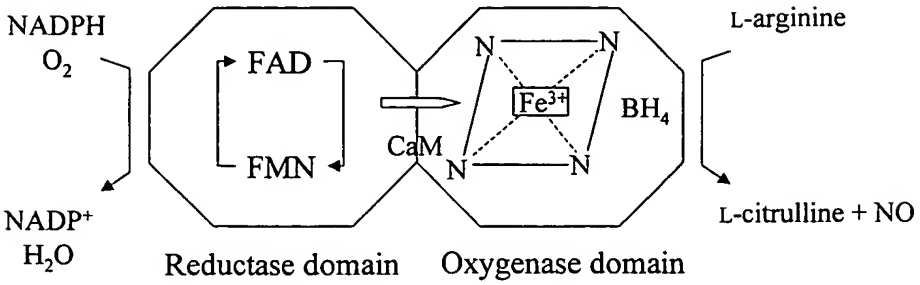


Fig. 7. Schematic representation of the nitric oxide synthase monomer. Nitric oxide synthases are dimers each of whose monomers is composed of two domains; they use three substrates: L-arginine, NADPH and O_2, and require five cofactors: FAD, FMN, calmodulin (CaM), tetrahydrobiopterin (BH_4) and heme.

the presence of numerous inducible Type-II NOS immunoreactive microglia. This excessive vascular NO production can generate peroxynitrite by reaction with O_2^-, which is also increased. Such a pathway for peroxynitrite formation may be postulated in cerebral amyloid angiopathy, which is frequently associated with AD [84,93].

3.4. By Type-I NOS activity in arginine- or tetrahydrobiopterin-depleted conditions

Type-I NOS and Type-II NOS catalyze electron transfer from NADPH to O_2 in the first step of the NO-production mechanism (Fig. 7). At low arginine concentrations [62,67], however, Type-II NOS strongly reduces the NADPH oxidation rate, while Type-I NOS still oxidizes NADPH with an unmodified rate and then reduces O_2 to give O_2^-.

Using electron paramagnetic resonance spin trapping techniques, Xia et at. [91] monitored NO and O_2^- formation in intact cells. These authors, found a 20-fold increase of O_2^- formation in Type-I NOS-transfected human kidney 293 cells as the cytosolic L-arginine levels decreased from 70.4 to 15.8 μM, while there was only a four-fold decline in NO generation. The NOS inhibitor *N*-nitro-L-arginine methyl ester virtually abolished the O_2^- formation. Therefore, at low arginine concentrations, Type-I NOS produces simultaneously O_2^- and NO at the same site and may act as an efficient peroxynitrite synthase.

A similar conclusion was obtained by Gorren and Mayer [32] in a study of Type-I NOS activity as a function of tetrahydrobiopterin (BH_4) concentration. Type-I NOS is a dimeric enzyme which exhibits strong anti-cooperative binding of BH_4. The dissociation constant of BH_4 to the first subunit was estimated [31] to be less than 1 nM, whereas the dissociation constant of the second BH_4 molecule was greater than 1 μM. At very low BH_4 concentrations ($< 10^{-9}$ M), no BH_4 molecule binds Type-I NOS and O_2^- is produced; whereas at high BH_4 concentrations ($> 10^{-6}$ M), two BH_4 molecules bind the enzyme and NO is produced. For BH_4 concentrations in between the two dissociation constants values, only one BH_4 molecule binds the enzyme, both subunits function independently and peroxynitrite is produced.

4. Relevance of peroxynitrite to neurodegenerative disorders

Toxicity of peroxynitrite has been reported for cultured cells such as rat thymocytes [69,70], neurons (peroxynitrite acting as an inhibitor of glutamate uptake) [85,86] and PC12 cells (large doses of peroxynitrite resulted in cell necrosis, whereas lower concentrations induced apoptosis) [22].

Peroxynitrite has also been suggested to contribute to tissue damage in AD, PD, MS, ALS and the parkinsonism dementia complex of Guam.

4.1. Amyotrophic lateral sclerosis

As early as 1993, Beckman et al. [8] pointed out that mutations in SOD associated with the autosomal dominant inheritance of familial ALS could both double the steady state concentration of O_2^- by decreasing SOD activity by 50% and increase nitration of critical cellular targets by allowing a greater access of peroxynitrite to copper, a strong tyrosyl nitration catalysis, in the SOD active site. The NO required for peroxynitrite synthesis was assumed to be produced by inter neurons surrounding the motor neuron that innervates the muscle fiber. More recently [19], this hypothesis was strengthened by the demonstration that the zinc affinity of four ALS-associated SOD mutants was decreased up to 30-fold compared with wild-type SOD. These investigators also showed that the motor neuron protein neurofilament-L (NF-L) could bind zinc atoms with sufficient affinity to potentially remove zinc from SOD and that the loss of zinc from wild-type SOD almost doubled the efficiency of this enzyme for catalyzing for peroxynitrite-mediated tyrosine nitration. By immunohistochemistry, Abe et al. [1,2] and Chou et al. [13,14] detected more nitration in motor neurons of ALS

than in controls. This suggests an up regulation of the nitration of protein–tyrosine residues in motor neurons of the spinal cord in ALS. However, Strong et al. [81] recently reported that there were no significant qualitative or quantitative modifications in the nitrotyrosine-immunoreactivity of NF-L isolated from sporadic ALS cervical spinal cord tissue as compared with age-matched non-ALS controls. The low fraction of motor neurons in all neurons in spinal cord could account for these opposite results.

4.2. Multiple sclerosis

In the CNS of animals with experimental allergic encephalomyelitis (EAE) and in patients with MS, several findings suggest a potential role of peroxynitrite in the pathogenesis of demyelinating lesions. The presence of NO has been demonstrated in the spinal cord of mice with EAE by Lin et al. [51], Type-II NOS mRNA has been detected in EAE models by Cross et al. [18] and Merrill et al. [58] observed in vitro an enhanced production by microglial cells of NO, which damages myelin and oligodendrocytes. More recently, Cross et al. [17] provided evidence for the production of peroxynitrite in CNS tissues from EAE mice by immunocytochemical detection of nitrotyrosine.

In MS patients, Sarchielli et al. [71] reported an overexpression of the proinflammatory cytokines IL1-β, IL2, IFNγ and TNF and a defective production of the anti-inflammatory cytokine TGF-β associated with an increase in Type-II NOS mRNA expression and nitrite production in peripheral blood mononuclear cells. This increased secretion during homing of monocytes in the CNS could contribute to damage of the blood–brain barrier by means of peroxynitrite formation. This assumption is strengthened by recent results showing elevated levels of nitrite and nitrates in cerebrospinal fluid in the extreme stage of MS exacerbation [26–28,92]. Moreover, Gurwitz and Kloog [33] explained the elevated glutamate and aspartate levels in the cerebrospinal fluid of MS patients by peroxynitrite generation.

4.3. Alzheimer's disease

The autotoxic loop hypothesis which assumes a direct relation between oxidative stress and neuron damage has been proposed as a potential pathogenic mechanism in AD [56,57]. Because peroxynitrite formation is an essential mechanism of oxidative damage and because it leads to the nitration of tyrosyl residues in proteins, attention has been focused on the immunocytochemical detection of nitrosyl residues in the CNS of AD patients.

Good et al. [30] demonstrated the presence of nitrotyrosyl residues in neurofibrillary tangles of AD brains but not in controls lacking such insoluble aggregates. These results were extended by Smith et al. [78] to the neuronal cytoplasm of neurodegenerative regions.

Hensley et al. [34] quantified, by means of HPLC with electrochemical array detection, the nitrotyrosyl content in the hyppocampus, neurocortical regions and ventricular cerebrospinal fluid. These authors measured five- to eight-fold more nitrotyrosine in AD patients than in cognitively normal subjects.

These results confirmed that oxidative stress is an important contributor to AD and suggested that peroxynitrite is directly implicated in the neuronal loss which is a prominent feature of AD. To clarify the relationship between protein nitration, neuron degeneration and DNA damage in AD, Su et al. [83] compared DNA strand breaking (detected by the deoxynucleotidyl transferase histochemical technique, TdT) and tyrosine-nitrated proteins (detected by immunocytochemistry using antibodies direct against nitrotyrosine) in the visual cortex from AD and control subjects. They demonstrated that the majority of TdT-labeled nuclei are associated with neurons exhibiting an up-regulation of nitrotyrosine expression. They concluded that, in AD, peroxynitrite can both promote DNA fragmentation by oxidative damage and prevent protein phosphorylation by tyrosine nitration, thus disturbing signal transduction mediated by tyrosine kinases.

Peroxynitrite thus appears as strongly implicated in neuronal cell death by necrotic and apoptotic processes in AD.

4.4. Parkinson's disease

PD is associated predominantly with degeneration of the cells in the substantia nigra, dopamine deficiency, loss of glutathione and Lewy body appearance. Post mortem studies undertaken in brain from PD patients have shown that oxidative damage is present and suggest that reactive oxygen species-mediated processes are important elements of the cell degeneration [39,40,52]. A model of parkinsonism can be provided by intraperitoneal administration to mice of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective neurotoxin of dopaminergic nigrastriatal neurons. Following MPTP administration, microglial cell activation, astrocytosis [44], impairment of mitochondrial function [59,77] and generation of reactive oxygen species [73] were observed, suggesting a possible generation of peroxynitrite.

Recent results add weight to the concept that peroxynitrite plays a pivotal role in the pathogenesis of PD. Good et al. [29] demonstrated that antibodies to nitrotyrosine stain the core but not the halo of Lewy bodies in serotonergic neurons of PD patients. Ara et al. [3] observed the inactivation of tyrosine hydroxylase by nitration following exposure of PC 12 cells to either peroxynitrite or MPTP. Moreover, these authors observed a nitration of tyrosine residues in tyrosyl hydroxylase that parallels the decline in dopamine levels in mouse striatum after MTPT administration. Beal [7] reported the protective effect of Type-I NOS inhibitors on the neurotoxicity of MPTP in both mice and

primates. Barker et al. [5] demonstrated the susceptibility of glutathione reductase, the enzyme which regenerates glutathione from its oxidized form, to peroxynitrite. An apparent 50% inhibition occurred at a peroxynitrite concentration of 0.09 mM. Since loss of intracellular glutathione from substantia nigra is considered to be an early event in PD, these data suggest that peroxynitrite could contribute depleting the cells of a major antioxidant defense rendering the nigrostriatal pathway susceptible to toxic insult.

Taken together, these results strongly suggest that peroxynitrite might be involved in PD pathogenesis.

5. Peroxynitrite as a mediator of neuronal damage

The association of many inflammatory mediators with the lesions detected in the brain of patients with neurodegenerative disorders such as AD, PD, ALS and MS has led Mc Geer and Mc Geer [56,57] to assume that the neuronal damage observed in these diseases is caused by a chronic innate immune reaction in brain. In the autotoxic loop model proposed by these authors, the inflammatory reaction is not believed to be the primary cause of the neuronal damage but only the autotoxic response to an unknown primary neuronal insult.

Current evidence enables us to consider that peroxynitrite, the strong oxidizing and nitrating agent formed by the fast reaction between NO and O_2^- has the potential to both initiate and sustain the autotoxic loop considered as a neuronal damaging mechanism in neurodegenerative diseases.

Under normal conditions, neurons produce NO, via Type-I NOS, as an intracellular messenger which has an important role in synaptic plasticity. Shibuki and Okada [76] have shown that 70–100 nM NO was produced in cerebellum slices after brief electrical stimulations. Since phagocytic cells are in a resting and an immunodepressed state, the intracellular concentrations of O_2^- are very low and only non-significant amounts of peroxynitrite can be formed.

On the contrary, under either L-arginine- or BH_4-depleted conditions, Type-I NOS monomers can function independently of each other. The enzyme generates peroxynitrite instead of NO and can promote some neuronal damage with deposition of cellular debris.

This primary neuronal insult can activate microglial cells and initiate the second step of the neurodegenerative process by induction of Type-II NOS, which produces large amounts of NO, and stimulation of NADPH-oxidase, which produces O_2^-. As experimentally demonstrated by Ischiropoulos et al. [38] in a study of rat alveolar macrophages activated with phorbol ester, NO becomes the major target of O_2^- to produce peroxynitrite. This second step corresponds to the initiation of the autotoxic loop, which amplifies the inflammatory reaction, increases the amounts of peroxynitrite generated and leads to further neuronal damage.

Type-III NOS expressed in microvessels and motor neurons can also generate NO for peroxynitrite formation. The studies of Estèvez et al. [23–25] on embryonic rat spinal motor neurons suggest a mechanism for peroxynitrite inducing cell death which is unrelated to the inflammation model. These authors observed an expression of Type-III NOS but not of Type-I NOS by motor neurons cultured with brain-derived neurotrophic factor (BDNF). NO generated showed a protective activity on cell viability, through activation of soluble guanylate cyclase and stimulation of cGMP synthesis. On the contrary, trophic factor deprivation promoted Type-I NOS expression and cell death by apoptosis. This motor neuron degeneration was promoted by NO produced by Type-I NOS and reversed by SOD, suggesting that formation of peroxynitrite initiates apoptosis. These data suggest that, in motor neurons, peroxynitrite formation can be induced by trophic factor deprivation. This highlights a new pathway for peroxynitrite in vivo generation mediated by the absence of cell signaling events. The formation of peroxynitrite by this pathway can initiate the autotoxic loop and lead to neurodegeneration by the mechanism we described previously for generation of peroxynitrite by Type-I NOS under L-arginine- or BH_4-depleted conditions.

With its strong oxidizing and nitrating properties, peroxynitrite could thus play a pivotal role not only in the exacerbation of the neuronal damage in the brain of patients with neurodegenerative disorders but also in the initiation process of these pathologies.

Acknowledgements

The authors thank Dr. Sharon Lynn Salhi for critical comments and help in preparing the manuscript.

References

[1] K. Abe, L.H. Pan, M. Watanabe, T. Kato, Y. Itoyama, Induction of nitrotyrosine-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis, Neurosci. Lett. 199 (1995) 152–154.
[2] K. Abe, L.H. Pan, M. Watanabe, H. Konno, T. Kato, Y. Itoyama, Upregulation of protein–tyrosine nitration in the anterior horn cells of amyotrophic lateral sclerosis, Neurol. Res. 19 (1997) 124–128.
[3] J. Ara, S. Przedborski, A.B. Naini, V. Jackson-Lewis, R.R. Trifiletti, J. Horwitz, H. Ischiropoulos, Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Proc. Natl. Acad. Sci. USA 95 (1998) 7659–7663.
[4] S.W. Barger, A.D. Harmon, Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E, Nature 388 (1997) 878–881.
[5] J.E. Barker, S.J. Heales, A. Cassidy, J.P. Bolanos, J.M. Land, J.B. Clark, Depletion of brain glutathione results in a decrease of glu-

tathione reductase activity; an enzyme susceptible to oxidative damage, Brain Res. 716 (1996) 118–122.
[6] G. Bartosz, Peroxynitrite: mediator of the toxic action of nitric oxide, Acta Biochim. Pol. 43 (1996) 645–659.
[7] M.F. Beal, Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis, Ann. Neurol. 44 (1998) S110–114.
[8] J.S. Beckman, M. Carson, C.D. Smith, W.H. Koppenol, ALS, SOD and peroxynitrite, Nature 364 (1993) 584, letter.
[9] U. Beffert, M. Danik, P. Kryzwkowski, C. Ramassamy, F. Berrada, J. Poirier, The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease, Brain Res. Rev. 27 (1998) 119–142.
[10] E. Bonfoco, D. Krainc, M. Ankarcrona, P. Nicotera, S.A. Lipton, Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures, Proc. Natl. Acad. Sci. USA 92 (1995) 7162–7166.
[11] N.Y. Calingasan, L.C. Park, L.L. Calo, R.R. Trifiletti, S.E. Gandy, G.E. Gibson, Induction of nitric oxide synthase and microglial responses precede selective cell death induced by chronic impairment of oxidative metabolism, Am. J. Pathol. 153 (1998) 599–610.
[12] A. Cassina, R. Radi, Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport, Arch. Biochem. Biophys. 328 (1996) 309–316.
[13] S.M. Chou, H.S. Wang, K. Komai, Colocalization of NOS and SOD1 in neurofilament accumulation within motor neurons of amyotrophic lateral sclerosis: an immunohistochemical study, J. Chem. Neuroanat. 10 (1996) 249–258.
[14] S.M. Chou, H.S. Wang, A. Taniguchi, Role of SOD-1 and nitric oxide/cyclic GMP cascade on neurofilament aggregation in ALS/MND, J. Neurol. Sci. 139 (1996) 16–26, Suppl.
[15] C. Colton, S. Wilt, D. Gilbert, O. Chernyshev, J. Snell, M. Dubois-Dalcq, Species differences in the generation of reactive oxygen species by microglia, Mol. Chem. Neuropathol. 28 (1996) 15–20.
[16] B. Connor, M. Dragunow, The role of neuronal growth factors in neurodegenerative disorders of the human brain, Brain Res. Rev. 27 (1998) 1–39.
[17] A.H. Cross, P.T. Manning, M.K. Stern, T.P. Misko, Evidence for the production of peroxynitrite in inflammatory CNS demyelination, J. Neuroimmunol. 80 (1997) 121–130.
[18] A.H. Cross, T.P. Misko, R.F. Lin, W.F. Hickey, J.L. Trotter, R.G. Tilton, Aminoguanidine, an inhibitor of inducible nitric oxide synthase, ameliorates experimental autoimmune encephalomyelitis in SJL mice, J. Clin. Invest. 93 (1994) 2684–2690.
[19] J.P. Crow, J.B. Sampson, Y. Zhuang, J.A. Thompson, J.S. Beckman, Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite, J. Neurochem. 69 (1997) 1936–1944.
[20] A.T. Demiryurek, I. Cakici, I. Kanzik, Peroxynitrite: a putative cytotoxin, Pharmacol. Toxicol. 82 (1998) 113–117.
[21] J.L. Dinerman, T.M. Dawson, M.J. Schell, A. Snowman, S.H. Snyder, Endothelial nitric oxide synthase localized to hippocampal pyramidal cells: implications for synpatic plasticity, Proc. Natl. Acad. Sci. USA 91 (1994) 4214–4218.
[22] A.G. Estévez, R. Radi, L. Barbeito, J.T. Shin, J.A. Thompson, J.S. Beckman, Peroxynitrite-induced cytotoxicity in PC12 cells: evidence for an apoptotic mechanism differentially modulated by neurotrophic factors, J. Neurochem. 65 (1995) 1543–1550.
[23] A.G. Estévez, N. Spear, S.M. Manuel, L. Barbeito, R. Radi, J.S. Beckman, Role of endogenous nitric oxide and peroxynitrite formation in the survival and death of motor neurons in culture, Prog. Brain Res. 118 (1998) 269–280.
[24] A.G. Estévez, N. Spear, S.M. Manuel, R. Radi, C.E. Henderson, L. Barbeito, J.S. Beckman, Nitric oxide and superoxide contribute to motor neuron apoptosis induced by trophic factor deprivation, J. Neurosci. 18 (1998) 923–931.
[25] A.G. Estévez, N. Spear, J.A. Thompson, T.L. Cornwell, R. Radi, L. Barbeito, J.S. Beckman, Nitric oxide-dependent production of cGMP supports the survival of rat embryonic motor neurons cultured with brain-derived neurotrophic factor, J. Neurosci. 18 (1998) 3708–3714.
[26] G. Giovannoni, Cerebrospinal fluid and serum nitric oxide metabolites in patients with multiple sclerosis, Mult. Scler. 4 (1998) 27–30.
[27] G. Giovannoni, S.J. Heales, J.M. Land, E.J. Thompson, The potential role of nitric oxide in multiple sclerosis, Mult. Scler. 4 (1998) 212–216.
[28] G. Giovannoni, R.F. Miller, S.J. Heales, J.M. Land, M.J. Harrison, E.J. Thompson, Elevated cerebrospinal fluid and serum nitrate and nitrite levels in patients with central nervous system complications of HIV-1 infection: a correlation with blood–brain-barrier dysfunction, J. Neurol. Sci. 156 (1998) 53–58.
[29] P.F. Good, A. Hsu, P. Werner, D.P. Perl, C.W. Olanow, Protein nitration in Parkinson's disease, J. Neuropathol. Exp. Neurol. 57 (1998) 338–342.
[30] P.F. Good, P. Werner, A. Hsu, C.W. Olanow, D.P. Perl, Evidence of neuronal oxidative damage in Alzheimer's disease, Am. J. Pathol. 149 (1996) 21–28.
[31] A.C. Gorren, B.M. List, A. Schrammel, E. Pitters, B. Hemmens, E.R. Werner, K. Schmidt, B. Mayer, Tetrahydrobiopterin-free neuronal nitric oxide synthase: evidence for two identical highly anticooperative pteridine binding sites, Biochemistry 35 (1996) 16735–16745.
[32] A.C. Gorren, B. Mayer, The versatile and complex enzymology of nitric oxide synthase, Biochemistry (Moscow) 63 (1998) 734–743.
[33] D. Gurwitz, Y. Kloog, Peroxynitrite generation might explain elevated glutamate and aspartate levels in multiple sclerosis cerebrospinal fluid, Eur. J. Clin. Invest. 28 (1998) 760–761, letter.
[34] K. Hensley, M.L. Maidt, Z. Yu, H. Sang, W.R. Markesbery, R.A. Floyd, Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation, J. Neurosci. 18 (1998) 8126–8132.
[35] J. Hu, K.T. Akama, G.A. Krafft, B.A. Chromy, L.J. Van Eldik, Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release, Brain Res. 785 (1998) 195–206.
[36] R.E. Huie, S. Padmaja, The reaction of NO with superoxide, Free Radical Res. Commun. 18 (1993) 195–199.
[37] M. Ii, M. Sunamoto, K. Ohnishi, Y. Ichimori, beta-amyloid protein-dependent nitric oxide production from microglial cells and neurotoxicity, Brain Res. 720 (1996) 93–100.
[38] H. Ischiropoulos, L. Zhu, J.S. Beckman, Peroxynitrite formation from macrophage-derived nitric oxide, Arch. Biochem. Biophys. 298 (1992) 446–451.
[39] P. Jenner, C.W. Olanow, Oxidative stress and the pathogenesis of Parkinson's disease, Neurology 47 (1996) S161–170.
[40] P. Jenner, C.W. Olanow, Understanding cell death in Parkinson's disease, Ann. Neurol. 44 (1998) S72–84.
[41] D.B. Kantor, M. Lanzrein, S.J. Stary, G.M. Sandoval, W.B. Smith, B.M. Sullivan, N. Davidson, E.M. Schuman, A role for endothelial NO synthase in LTP revealed by adenovirus-mediated inhibition and rescue, Science 274 (1996) 1744–1748.
[42] M. Kelm, R. Dahmann, D. Wink, M. Feelisch, The nitric oxide/superoxide assay. Insights into the biological chemistry of the NO/O_2^- interaction, J. Biol. Chem. 272 (1997) 9922–9932.
[43] J.F. Kerwin Jr., M. Heller, The arginine–nitric oxide pathway: a target for new drugs, Med. Res. Rev. 14 (1994) 23–74.
[44] M. Kohutnicka, E. Lewandowska, I. Kurkowska-Jastrzebska, A. Czlonkowski, A. Czlonkowska, Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Immunopharmacology 39 (1998) 167–180.
[45] E.H. Koo, S.L. Squazzo, D.J. Selkoe, C.H. Koo, Trafficking of cell-surface amyloid beta-protein precursor: I. Secretion, endocytosis and recycling as detected by labeled monoclonal antibody, J. Cell Sci. 109 (1996) 991–998.

[46] W.H. Koppenol, J.J. Moreno, W.A. Pryor, H. Ischiropoulos, J.S. Beckman, Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide, Chem. Res. Toxicol. 5 (1992) 834–842.
[47] G.W. Kreutzberg, Microglia, the first line of defence in brain pathologies, Arzneimittelforschung 45 (1995) 357–360.
[48] K.D. Kroncke, K. Fehsel, V. Kolb-Bachofen, Nitric oxide: cytotoxicity versus cytoprotection — how, why, when, and where?, Nitric Oxide 1 (1997) 107–120.
[49] W. Le, W.J. Xie, O. Nyormoi, B.K. Ho, R.G. Smith, S.H. Appel, beta-Amyloidl-40 increases expression of beta-amyloid precursor protein in neuronal hybrid cells, J. Neurochem. 65 (1995) 2373–2376.
[50] W.D. Le, L.V. Colom, W.J. Xie, R.G. Smith, M. Alexianu, S.H. Appel, Cell death induced by beta-amyloid 1–40 in MES 23.5 hybrid clone: the role of nitric oxide and NMDA-gated channel activation leading to apoptosis, Brain Res. 686 (1995) 49–60.
[51] R.F. Lin, T.S. Lin, R.G. Tilton, A.H. Cross, Nitric oxide localized to spinal cords of mice with experimental allergic encephalomyelitis: an electron paramagnetic resonance study, J. Exp. Med. 178 (1993) 643–648.
[52] L. Lyras, R.H. Perry, E.K. Perry, P.G. Ince, A. Jenner, P. Jenner, B. Halliwell, Oxidative damage to proteins, lipids, and DNA in cortical brain regions from patients with dementia with Lewy bodies, J. Neurochem. 71 (1998) 302–312.
[53] J. MacMicking, Q.W. Xie, C. Nathan, Nitric oxide and macrophage function, Annu. Rev. Immunol. 15 (1997) 323–350.
[54] F. Mastrogiacomo, C. Bergeron, S.J. Kish, Brain alpha-ketoglutarate dehydrogenase complex activity in Alzheimer's disease, J. Neurochem. 61 (1993) 2007–2014.
[55] M.P. Mattson, B. Cheng, D. Davis, K. Bryant, I. Lieberburg, R.E. Rydel, beta-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity, J. Neurosci. 12 (1992) 376–389.
[56] E.G. McGeer, P.L. McGeer, The importance of inflammatory mechanisms in Alzheimer disease, Exp. Gerontol. 33 (1998) 371–378.
[57] P.L. McGeer, E.G. McGeer, The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases, Brain Res. Rev. 21 (1995) 195–218.
[58] J.E. Merrill, D.H. Kono, J. Clayton, D.G. Ando, D.R. Hinton, F.M. Hofman, Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice, Proc. Natl. Acad. Sci. USA 89 (1992) 574–578, published erratum appears in Proc. Natl. Acad. Sci. (USA) 1992 Nov 1; 89 (21): 10562.
[59] Y. Mizuno, H. Yoshino, S. Ikebe, N. Hattori, T. Kobayashi, S. Shimoda-Matsubayashi, H. Matsumine, T. Kondo, Mitochondrial dysfunction in Parkinson's disease, Ann. Neurol. 44 (1998) S99–109.
[60] M.P. Murphy, M.A. Packer, J.L. Scarlett, S.W. Martin, Peroxynitrite: a biologically significant oxidant, Gen. Pharmacol. 31 (1998) 179–186.
[61] S. Nakanishi, Y. Nakajima, M. Masu, Y. Ueda, K. Nakahara, D. Watanabe, S. Yamaguchi, S. Kawabata, M. Okada, Glumamate receptors: brain function and signal transduction, Brain Res. Rev. 26 (1998) 230–235.
[62] C. Nathan, Q.W. Xie, Nitric oxide synthases: roles, tolls, and controls, Cell 78 (1994) 915–918.
[63] L. Nilsson, J. Rogers, H. Potter, The essential role of inflammation and induced gene expression in the pathogenic pathway of Alzheimer's disease, Front. Biosci. 3 (1998) d436–446.
[64] T.J. O'Dell, P.L. Huang, T.M. Dawson, J.L. Dinerman, S.H. Snyder, E.R. Kandel, M.C. Fishman, Endothelial NOS and the blockade of LTP by NOS inhibitors in mice lacking neuronal NOS, Science 265 (1994) 542–546.
[65] M. Okabe, S. Saito, T. Saito, K. Ito, S. Kimura, T. Niioka, M. Kurasaki, Histochemical localization of superoxide dismutase activity in rat brain, Free Radical Biol. Med. 24 (1998) 1470–1476, In Process Citation.
[66] D. Paris, T.A. Parker, T. Town, Z. Suo, C. Fang, J. Humphrey, F. Crawford, M. Mullan, Role of peroxynitrite the vasoactive and cytotoxic effects of Alzheimer's beta-amyloidl-40 peptide, Exp. Neurol. 152 (1998) 116–122.
[67] S. Pou, W.S. Pou, D.S. Bredt, S.H. Snyder, G.M. Rosen, Generation of superoxide by purified brain nitric oxide synthase, J. Biol. Chem. 267 (1992) 24173–24176.
[68] A. Saha, S. Goldstein, D. Cabelli, G. Czapski, Determination of optimal conditions for synthesis of peroxynitrite by mixing acidified hydrogen peroxide with nitrite, Free Radical Biol. Med. 24 (1998) 653–659.
[69] M.G. Salgo, E. Bermudez, G.L. Squadrito, W.A. Pryor, Peroxynitrite causes DNA damage and oxidation of thiols in rat thymocytes, Arch. Biochem. Biophys. 322 (1995) 500–505, corrected, published erratum appears in Arch. Biochem. Biophys. 1995 Dec 1; 324 (1) 200.
[70] M.G. Salgo, G.L. Squadrito, W.A. Pryor, Peroxynitrite causes apoptosis in rat thymocytes, Biochem. Biophys. Res. Commun. 215 (1995) 1111–1118.
[71] P. Sarchielli, A. Orlacchio, F. Vicinanza, G.P. Pelliccioli, M. Tognoloni, C. Saccardi, V. Gallai, Cytokine secretion and nitric oxide production by mononuclear cells of patients with multiple sclerosis, J. Neuroimmunol. 80 (1997) 76–86.
[72] J.B. Schulz, R.T. Matthews, T. Klockgether, J. Dichgans, M.F. Beal, The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative diseases, Mol. Cell Biochem. 174 (1997) 193–197.
[73] K.P. Scotcher, I. Irwin, L.E. DeLanney, J.W. Langston, D. Di Monte, Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on ATP levels of mouse brain synaptosomes, J. Neurochem. 54 (1990) 1295–1301.
[74] B. Sengpiel, E. Preis, J. Krieglstein, J.H. Prehn, NMDA-induced superoxide production and neurotoxicity in cultured rat hippocampal neurons: role of mitochondria, Eur. J. Neurosci. 10 (1998) 1903–1910.
[75] K.F. Sheu, A.J. Cooper, K. Koike, M. Koike, J.G. Lindsay, J.P. Blass, Abnormality of the alpha-ketoglutarate dehydrogenase complex in fibroblasts from familial Alzheimer's disease, Ann. Neurol. 35 (1994) 312–318.
[76] K. Shibuki, D. Okada, Endogeneous nitric oxide release required for long-term synaptic depression in the cerebellum, Nature 349 (1991) 326–328.
[77] T.P. Singer, N. Castagnoli Jr., R.R. Ramsay, A.J. Trevor, Biochemical events in the development of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, J. Neurochem. 49 (1987) 1–8.
[78] M.A. Smith, P.L. Richey Harris, L.M. Sayre, J.S. Beckman, G. Perry, Widespread peroxynitrite-mediated damage in Alzheimer's disease, J. Neurosci. 17 (1997) 2653–2657.
[79] G.L. Squadrito, W.A. Pryor, Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide, Free Radical Biol. Med. 25 (1998) 392–403.
[80] P.F. Stahel, M.C. Morganti-Kossmann, T. Kossmann, The role of the complement system in traumatic brain injury, Brain Res. Rev. 27 (1998) 243–256.
[81] M.J. Strong, M.M. Sopper, J.P. Crow, W.L. Strong, J.S. Beckman, Nitration of the low molecular weight neurofilament is equivalent in sporadic amyotrophic lateral sclerosis and control cervical spinal cord, Biochem. Biophys. Res. Commun. 248 (1998) 157–164.
[82] C. Sturchler-Pierrat, D. Abramowski, M. Duke, K.H. Wiederhold, C. Mistl, S. Rothacher, B. Ledermann, K. Burki, P. Frey, P.A. Paganetti, C. Waridel, M.E. Calhoun, M. Jucker, A. Probst, M. Staufenbiel, B. Sommer, Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology, Proc. Natl. Acad. Sci. USA 94 (1997) 13287–13292.
[83] J.H. Su, G. Deng, C.W. Cotman, Neuronal DNA damage precedes tangle formation and is associated with up- regulation of nitrotyrosine in Alzheimer's disease brain, Brain Res. 774 (1997) 193–199.

[84] Z. Suo, J. Tan, A. Placzek, F. Crawford, C. Fang, M. Mullan, Alzheimer's beta-amyloid peptides induce inflammatory cascade in human vascular cells: the roles of cytokines and CD40, Brain Res. 807 (1998) 110–117.

[85] D. Trotti, N.C. Danbolt, A. Volterra, Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration?, Trends Pharmacol. Sci. 19 (1998) 328–334.

[86] D. Trotti, D. Rossi, O. Gjesdal, L.M. Levy, G. Racagni, N.C. Danbolt, A. Volterra, Peroxynitrite inhibits glutamate transporter subtypes, J. Biol. Chem. 271 (1996) 5976–5979.

[87] M.P. Vitek, J. Snell, H. Dawson, C.A. Colton, Modulation of nitric oxide production in human macrophages by apolipoprotein-E and amyloid-beta peptide, Biochem. Biophys. Res. Commun. 240 (1997) 391–394.

[88] Y. Vodovotz, M.S. Lucia, K.C. Flanders, L. Chesler, Q.W. Xie, T.W. Smith, J. Weidner, R. Mumford, R. Webber, C. Nathan, A.B. Roberts, C.F. Lippa, M.B. Sporn, Inducible nitric oxide synthase in tangle-bearing neurons of patients with Alzheimer's disease, J. Exp. Med. 184 (1996) 1425–1433.

[89] D.G. Walker, S.U. Kim, P.L. McGeer, Complement and cytokine gene expression in cultured microglial derived from postmortem human brains, J. Neurosci. Res. 40 (1995) 478–493.

[90] D.T. Weldon, S.D. Rogers, J.R. Ghilardi, M.P. Finke, J.P. Cleary, E. O'Hare, W.P. Esler, J.E. Maggio, P.W. Mantyh, Fibrillar beta-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo, J. Neurosci. 18 (1998) 2161–2173.

[91] Y. Xia, V.L. Dawson, T.M. Dawson, S.H. Snyder, J.L. Zweier, Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury, Proc. Natl. Acad. Sci. USA 93 (1996) 6770–6774.

[92] T. Yamashita, Y. Ando, K. Obayashi, M. Uchino, M. Ando, Changes in nitrite and nitrate (NO^{2-}/NO^{3-}) levels in cerebrospinal fluid of patients with multiple sclerosis, J. Neurol. Sci. 153 (1997) 32–34.

[93] C. Zarow, B. Zaias, S.A. Lyness, H. Chui, Cerebral amyloid angiopathy in Alzheimer disease is associated with apolipoprotein E4 and cortical neuron loss, Alzheimer Dis. Assoc. Disord. 13 (1999) 1–8, In Process Citation.

EXHIBIT F

Annu. Rev. Neurosci. 1982. 5:219–241

MULTIPLE SCLEROSIS

Guy M. McKhann

Department of Neurology, Johns Hopkins Hospital and Johns Hopkins School of Medicine, Baltimore, Maryland 21205 USA

Introduction

Multiple sclerosis is the leading cause of serious neurological disease in young and middle-aged adults in the United States and in Western Europe (Johnson et al 1979). In its classical form, the disease is characterized by acute exacerbations followed by spontaneous remissions. As the disease progresses, many patients have fewer "attacks" and are affected by a slowly progressive pattern of disease. There are variants in the clinical course, ranging from an acute, fulminating disease to a pattern of few attacks at intervals as long as years. The propensity for spontaneous remission and the changing patterns of the disease with time make evaluation of therapeutic attempts extremely difficult.

The gross pathology of the brain in multiple sclerosis is the presence of circumscribed areas, or plaques, of loss of myelin which have irregular borders and occur anywhere in the white matter. The characteristic microscopic features of these plaques are sparing of nerve cell bodies and axons, areas of myelin dissolution, and an accompanying astroglial proliferation. Thus, multiple sclerosis has been considered the prototype of a *demyelinative disease.*

In this review, I present the unique features of multiple sclerosis and then use this information to discuss possible mechanisms of disease and approaches to therapy.

Epidemiology

The clinical onset of multiple sclerosis is rare before the age of 15 and decreases in frequency after the age of 45 years. There is a marked variation in the incidence (number of new patients recorded during a defined period) and prevalence (total number of current cases of the disease) according to latitude (Kurland 1970, Kurtzke 1975, Alter 1980). In Northern latitudes

0147-006X/82/0301-0219$02.00

the prevalence is higher (e.g. Denmark: 64/100,000; Southeastern Norway: 80/100,000; Rochester, Minnesota: 64/100,000). In lower latitudes it is lower (e.g. Parma, Italy: 12/100,000; Israel: 4/100,000). Near the equator the disease is virtually nonexistent or not reported.

This North-South gradient can be observed within a single country. For example, the prevalence in Rochester, Minnesota (latitude 44° N) is 64/100,000, while in New Orleans (latitude 30° N) it is 6/100,000. In Great Britain this latitudinal correlation is even more striking, with Aberdeen (latitude 57°) having a prevalence of 144/100,000 and Cornwall (latitude 50°), 63/100,000. In addition, there are areas such as the Orkney Islands (latitude 59° N) where the prevalence, 125–150/100, is three times the expected rate. There is also an East-West gradient: the disease is far less common in Japanese than in Europeans living at similar latitudes.

A pertinent question is what happens to people who migrate from an area of high incidence to one of low incidence? In two populations, those migrating to an area of high incidence (Great Britain) and those migrating to an area of low incidence (Israel), studies suggest that those who migrate after adolescence carry with them the prevalence rate of their place of origin, whereas those migrating in childhood acquire the prevalence of that in their host country (Dean et al 1977, Alter et al 1978, Dean 1980, Alter 1980).

INTERPRETATION A possible interpretation is that susceptible individuals are exposed to and acquire the disease well before clinical onset and before age 12 to 15 years. The disease then presents after a significant latent period. This interpretation is an integral part of the "viral hypothesis" of multiple sclerosis.

There are, however, other possible interpretations. For example, early exposure may provide immunity, as is presumably the case with poliomyelitis. Alternatively, some environmental factor, other than an infectious agent, may vary with latitude such as diet, exposure to sunlight, or sociocultural status. This area of multiple sclerosis research has recently been reviewed (Alter 1980, Dean 1980, Kurtzke 1980).

Genetic Studies

The incidence of multiple sclerosis is 15–20 times higher in relatives of patients than in the general population; the highest incidence is in siblings and the next highest in parents. The concordance in identical twins is higher than in fraternal twins. The concept of a genetic factor is strengthened by studies relating to leukocyte functions regulated by the sixth human chromosome. In the United States and Western Europe, the frequency of HLA antigens A3 and B7 are increased, as are B cell antigens DRw2 and DRw3. The strongest association is with DRw2, which occurs three to four

times more frequently in patients with multiple sclerosis than in the general population (Oger & Arnason 1980).

INTERPRETATION. The data support the hypothesis that there is a genetic component or a genetically determined susceptibility to some exogenous factor operative in multiple sclerosis.

Pathology

The characteristic pathology of multiple sclerosis is scattered plaques of demyelination of various ages. Histologically, these plaques show (*a*) sparing of nerve cell bodies and axons, except in older lesions where axons may be lost, (*b*) areas of myelin dissolution and the presence of macrophages containing myelin breakdown components around the periphery, (*c*) a loss of oligodendria, particularly in the center of plaques, (*d*) an intense astroglial response, and (*e*) perivascular cuffs of lymphocytes. Plaques can occur anywhere in central white matter, but have a predilection for optic nerve, brainstem, spinal cord, and the periventricular areas around the lateral ventricles. The correlation between the distribution and number of plaques and recorded clinical symptoms and signs is often quite poor.

Because of the chronicity of the disease, analysis of older plaques does not reveal the cellular events associated with the development of acute lesions. Light and electron microscopic studies of acute plaques have indicated glial hyperplasia, often in a ring around the plaque. The cells participating in this hyperplasia are astrocytes, microglia, and perhaps oligodendroglia. Inclusion-bearing cells may occur at the edge of acute plaques; these could be either altered oligodendroglia or a form of undifferentiated neuroglial cell. The nature of the inclusion material is not known; it has been suggested by some to represent viral antigen and by others to reflect storage of material by these cells. Another feature of early plaques is the degree of perivascular infiltration with mononuclear cells, often extending some distance away from the plaque. Associated with this infiltration there may be edema of the surrounding brain tissue (Adams 1977, Prineas & Connell 1978).

Biochemical studies of involved areas show the changes one might expect in areas of glial proliferation and myelin breakdown, such as an increase in glial fibrillary astrocyte protein, an increase in lysosomal enzymes, and the presence of cholesterol esters around plaques. There is, however, a disproportionate loss of myelin basic protein and myelin-associated glycoproteins compared to other components of myelin (Itoyama et al 1980).

An important but unresolved question is whether the normal-appearing white matter is really normal. Both histological abnormalities (primarily a diffuse astrocytic proliferation) and biochemical abnormalities (consisting

of an increase in lysosomal enzymes, particularly, n-acetyl-B-D-glucosaminidase) have been reported (Allen 1980).

A major discrepancy between the pathology and clinical course of multiple sclerosis is the lack of evidence of a reparative process, specifically remyelination, as a basis for clinical remissions. In most plaques the majority of axons remain demyelinated, with remyelination limited to the periphery of lesions (Prineas & Connell 1979).

INTERPRETATION The pathological analysis indicates a selective disease process with loss of myelin and oligodendroglia, proliferation of astrocytes, microglia, and plasma cells, and relative sparing of axons. There is no convincing evidence of viral inclusions. The early cellular events are not known. The disease is still considered to be one with multiple foci of discrete demyelination. However, there remains the possibility that there is more generalized involvement of white matter or blood vessels. The latter point is important in terms of research strategy aimed at the study of autopsy tissue.

Cellular and Chemical Pathology

STUDIES WITH ISOLATED OLIGODENDROGLIA If demyelination is the primary pathological process, then it is logical to study the putative targets: oligodendroglia and myelin. Oligodendroglia can be obtained as relatively pure populations from a variety of species, including man (Poduslo & Norton 1972, Fewster & Blackstone 1975, Snyder et al 1980). These cells can be maintained in vitro and will synthesize lipids enriched in myelin, such as galactocerebrosides (Poduslo et al 1978, Szuchet et al 1980). In multiple sclerosis, fresh autopsy tissue is difficult to obtain under circumstances in which current cell isolation techniques can be applied. In addition, there is no methodology for obtaining oligodendroglia from only the periplaque areas. To date, the few preliminary studies that have been performed, have demonstrated no abnormalities in oligodendroglia isolated from patients who have died with multiple sclerosis.

An alternative approach has been to use oligodendroglia from some other species, such as the rat or cow, or from human abortus material, as a target for possible serum factors in multiple sclerosis. These studies are performed on either oligodendrocytes obtained by bulk isolation or on cultured, dispersed cells from cerebrum or corpus callosum. In the latter experimental situation, cell-specific antibodies, such as anti-galactocerebroside, are used to identify oligodendroglia (Raff et al 1978, 1979, Kennedy et al 1980, Schachner et al 1981). Abramsky et al (1977) reported that patients with multiple sclerosis had serum factors, presumably antibodies, that bound to the surface of oligodendroglia maintained in suspension cultures. Subse-

quent studies have indicated that sera from control patients are indistinguishable from those from patients with multiple sclerosis (Traugott et al 1979, Kennedy & Lisak 1979). In addition, using indirect immunofluorescence, it was observed that almost all sera contained immunoglobulins that bound weakly to human oligodendrocytes and fibroblasts, and to a lesser degree to Schwann cells and astrocytes (Kennedy & Lisak 1981).

It should be emphasized, however, that the methodologies for obtaining specific cell types from brain and for delineating cell-specific functions are undergoing rapid development and undoubtedly there will be efforts to apply these approaches to multiple sclerosis in the future.

STUDIES OF MYELIN There have been no consistent reports to indicate that myelin is abnormal in multiple sclerosis. Myelin is a relatively lipid-rich, protein-poor membrane. These characteristics allow it to be isolated by density gradient techniques. There have been several recent reviews of the morphology and biochemistry of myelination (Morell 1977, Palo 1978). Many of the components of myelin have been the subject of studies to determine whether a particular component is released into cerebrospinal fluid or blood at times of demyelination. Prior to the introduction of the radioimmunoassay and high pressure liquid chromatography, the insensitivity of analytical methods was a limiting factor for these studies. There is now evidence, however, that both myelin basic protein and myelin-associated glycoproteins are selectively decreased around plaques (Itoyama et al 1980). These latter observations have led to two lines of investigations: (*a*) the search in cerebrospinal fluid (CSF) and blood for myelin breakdown products, particularly myelin basic protein and peptides from that protein and (*b*) the study of proteases that might selectively attack myelin.

Myelin basic protein Several laboratories have reported the presence in CSF from persons with multiple sclerosis of material that reacts with antibodies raised against myelin basic protein (MBP) or its fragments (Cohen et al 1976, Whitaker 1977, Carson et al 1978, Trotter et al 1978). Studies from our laboratory indicate that this material is present during acute attacks and correlates with the duration of attack (Cohen et al 1980). The nature of the material in CSF is not entirely clear. One group has reported the presence of a molecule larger than basic protein (Carson et al 1980), while others have reported either the whole molecule (Cohen et al 1980) or a specific peptide fragment containing amino acids 43–88 (Whitaker et al 1980). In addition, it is possible to raise antibodies to purified basic protein that do not react with the material in CSF. Some of these discrepancies may be clarified when different laboratories use standardized monoclonal antibodies raised by the hybridoma technique (Kohler & Milstein 1976). Most

attempts to demonstrate myelin basic protein or its fragments in blood or serum have been negative, perhaps owing to the rapid clearance from blood of exogenously administered basic protein. However, fragments of myelin basic protein and antibodies to myelin basic protein have been recently demonstrated in sera of normals and of patients during acute exacerbations of multiple sclerosis (Paterson et al 1980). Further, these authors (Paterson et al 1981) have demonstrated that during acute attacks of multiple sclerosis, as in rats during the early stages of acute experimental allergic encephalitis (EAE), antibodies with a high affinity for MBP are found in sera; there is as well a corresponding decrease in free MBP fragments. This pattern of high affinity antibody with reduced levels of free MBP fragments is not seen in normals or in patients with quiescent disease. However, a full survey of patients with neurological diseases that are believed not to be of immunologic origin has not been completed (P. Y. Paterson, personal communication).

Attempts to correlate the presence of other components of myelin with the disease activity have been less successful. In our laboratories, the presence of lipids enriched in myelin, galactocerebroside, and sulfatide, and of a myelin-related enzyme, 2'-3' cyclic nucleotide 3'-phosphodiesterase (CNP), have not correlated with disease activity or with levels of myelin basic protein. Attempts are being made to develop sensitive radioimmunoassay for myelin-associated glycoproteins and proteolipid proteins and to determine possible correlation of these compounds with clinical disease.

Proteases in multiple sclerosis The study of proteases in multiple sclerosis is based, at least in part, on the sequence of cellular events in experimental allergic encephalomyelitis. In that disease, reactive cells, lymphocytes, and macrophages surround the myelin sheath, followed by a vesicular myelinolysis and a peeling off of myelin lamellae. A possible mechanism is the release from the reactive cells of specific proteases which attack myelin proteins, particularly myelin basic protein and myelin-associated glycoprotein. In multiple sclerosis, active stripping of myelin by macrophages has not been seen; however, close proximity of reactive cells (macrophages and microglia) to areas of myelin loss is seen. A sequence of events has been suggested in which macrophages are attracted to specific areas and then release neutral proteases which attack myelin basic protein (Bloom et al 1978). Recently, Bloom et al (1978) presented evidence that antimacrophage agents, such as silica, or proteinase inhibitors, such as *p*-nitrophenylguanidinobenzoate, pepstatin, or trans-4-aminomethylcyclohexane-1-carboxylic acid, will protect Lewis rats from experimental allergic encephalomyelitis. These findings suggest that macrophages play a role in the

clinical and histological expression of this experimental demyelinating disease (Brosnan et al 1980). Others have suggested that acid proteases, which might be of lysosomal origin from macrophages, lymphocytes or astrocytes, also selectively affect myelin basic protein. In CSF, both acidic and neutral proteinases have been found, both may vary with acute exacerbations (Richards & Cuzner 1978).

INTERPRETATION Several lines of evidence suggest that myelin basic protein and possibly myelin-associated glycoprotein are selectively affected in demyelination. A possible consequence is the release of fragments of basic protein into CSF during acute attacks of multiple sclerosis. Regardless of the mechanism, the presence in cerebrospinal fluid of myelin basic protein-like material provides an indication of activity of disease. The role of proteases in the process of demyelination is not clear.

Mechanism of Disease

The cause of multiple sclerosis is not known. A number of unique features of the disease have been used to suggest both an immunological mechanism and an infectious etiology. Both possible mechanisms are supported by experimental diseases in animals that have some of the features of multiple sclerosis. In recent years, as is discussed below, the distinction between the two mechanisms has become somewhat blurred.

IMMUNOLOGIC ABNORMALITIES IN MULTIPLE SCLEROSIS An immunological basis for multiple sclerosis has been suggested by the demonstration of a demyelinative disease, experimental allergic encephalomyelitis (EAE), produced by injecting animals with central nervous system antigens, particularly whole myelin, myelin basic protein, and galactocerebroside (Seil 1977). The acute form of EAE differs from multiple sclerosis in its pathology and its single-phase clinical course. Recently, a chronic, relapsing form of this disease has been produced which more closely resembles multiple sclerosis (Raine et al 1980). In this form of the disease, myelin basic protein may not be the responsible antigen (Wisniewski & Lassmann 1980).

Patients with multiple sclerosis have a number of immunologic abnormalities including (*a*) increased synthesis of immunoglobulins within the brain, (*b*) the possible presence of circulating factors which can cause demyelination or block neuroelectric transmission, and (*c*) decreased levels of lymphocyte subgroups, particularly T suppressor cells (Bloom 1980).

Immunoglobulins in CSF IgG is elevated in 70% of patients with clinically defined multiple sclerosis (MS). Subfractionation of these immunoglobulins by electrophoresis or isoelectric focusing has indicated separate

"oligoclonal bands" of immunoglobulin. Oligoclonal bands are present in the CSF in 80–90% of patients with MS, even those in which IgG may not be elevated. Once present, the bands persist. Recent observations suggest that the pattern of oligoclonal bands may change with repeated attacks of the disease (E. J. Thompson, personal communication). However, there is no specific pattern of oligoclonal bands that allows one to diagnose multiple sclerosis; each patient may have his or her own unique pattern (Laterre 1966, Link 1973, Thompson 1977). Analysis of individual plaques from the brains of a single patient indicates that different oligoclonal patterns are obtained from separate plaques, with only a few species of gamma globulin in common (Mattson et al 1980).

The source of the immunoglobulin in CSF of patients with multiple sclerosis is presumably plasma cells derived from B cell clones within brain parenchyma. As mentioned, the pattern of immunoglobulin extracted from individual plaques varies, suggesting that the patterns in cerebrospinal fluid is a composite from different brain regions. It is not at all clear to what antigens these centrally derived immunoglobulins are directed. Patients with multiple sclerosis have elevated titers to a number of viruses, particularly measles. However, an individual patient may have elevated titers to as many as four viruses (Vandvik & Norby 1980). Absorption of the CSF immunoglobulins with viral antigens does not selectively remove oligoclonal bands (Norby 1978). Similarly, antibodies to brain antigens, such as mylein basic proteins, when present, are at low levels and are not a significant proportion of the total CSF immunoglobulin fraction (Shorr et al 1981).

An alternative approach to relating the gamma globulin abnormalities in cereberospinal fluid to the mechanism of disease has been the characterization of CSF immunoglobulins by making anti-idiotypic antibodies (Ebers et al 1979, Naglekerken et al 1980). These antibodies, raised against the CSF IgG, would presumably bind to the combining site of CSF IgG. Possible antigens might then be used to displace the anti-idiotypic antibody. In such a study, anti-idiotypic antibodies have been raised against both CSF and serum IgG from patients with multiple sclerosis. Over a five-year period, up to 20% of the CSF IgG reacted with these anti-idiotypic antibodies. In this particular study, assays of these anti-idiotypic antibodies against CSF immunoglobulins of other patients with multiple sclerosis were negative in 12 of 13 patients, but did cross-react with one other patient (Baird et al 1980, Tachovsky & Baird 1980).

INTERPRETATION The antigenic determinants for the locally synthesized immunoglobulin in multiple sclerosis have not been defined. In other words, no "multiple sclerosis antigen" has been demonstrated. It is possible

that much of the immunoglobulin is the result of nonspecific activation of sequestered B cell clones; however, the analysis of immunoglobulin from isolated plaques and the binding of anti-idiotypic antibodies suggest that some IgG may be more specific. There remain the problems of identification of specific antibodies, determination of the associated antigens, and, finally, the demonstration of similar, if not identical, specificity in other patients with multiple sclerosis. The hope, obviously, is that this approach will indicate the antigenic nature of causal agent(s), as has been possible in chronic viral infections of the central nervous system, such as subacute sclerosing panencephalitis, in which similar immunologic responses occur.

CIRCULATING FACTORS THAT AFFECT MYELIN One approach to demonstrating a cell-specific abnormality in multiple sclerosis has been the search for circulating factors that affect myelin metabolism or alter oligodendroglial function. A number of laboratories have reported that 60–75% of MS patients have serum factors that will demyelinate myelinating explants (Bornstein 1963, Seil 1977). In some patients, there appears to be a correlation of the presence of serum demyelinative factors and disease activity. In contrast to EAE, the chemical nature of the demyelinating factors in MS sera has been difficult to define. The factor is sensitive to a number of manipulations such as freezing and thawing, heating, exposure to dialysis tubing or ammonium sulfate. In EAE sera, at least a portion of the demyelinating factor consists of IgG. On this basis, it has been assumed that the factor in MS sera is also IgG. However, adsorption of the sera with staphylococcus protein A, which removes IgG_1, IgG_2, and IgG_4, does not significantly decrease the demyelinating factor. Similarly, removal of IgA or IgM has no effect on demyelinating activity. Among immunoglobulins it is possible that the factor is IgG_3 (which is 5–7% of the total IgG fraction) or some other minor immunoglobulin such as IgD or IgE. On the other hand, it is possible that the demyelinating factor may not be an immunoglobulin at all (Grundke-Iqbal & Bornstein 1979).

INTERPRETATION One problem in interpretation of the significance of demyelinating factors in MS serum is the question of specificity to multiple sclerosis. Ten percent or more of the sera obtained from controls contain demyelinative factors. In amyotrophic lateral sclerosis, a disease in which the "target tissue" is the anterior horn cell, 50% of sera contain demyelinative factors. Thus, at the present time, it is difficult to assign a pathogenic role to an antimyelin or demyelinating factor in MS sera.

NEUROELECTRIC BLOCKING FACTORS Despite the observed pathology and the resulting historical emphasis on demyelination in multiple

sclerosis, there is evidence to suggest an alternative pathophysiological mechanism. The rapidity of appearance and disappearance of some clinical signs and symptoms in multiple sclerosis has suggested that demyelination and possible remyelination occurs too slowly to be the primary pathogenetic mechanism. Thus, the existence of rapidly acting serum factors that block nerve conduction has been proposed. The earliest indication that sera from patients with MS and from animals with EAE had neuroelectric blocking factors as well as a myelinotoxic factor resulted from the studies of Bornstein & Crain (1965). As with myelinotoxic factor, the specificity of possible neuroelectric factors soon came into question. Recent reports suggest, however, that one or more factors capable of blocking neuroelectric activity in invertebrate spinal cords are present in sera from MS patients and from animals with EAE. In MS the blocking factor(s) may correlate with clinical activity and may be within the IgG fraction (Schauf & Davis 1978). Much further work remains to be done in this area—in particular, serial studies in individual patients and further characterization of these factors.

CELLULAR RESPONSES The cellular responses in peripheral blood or CSF of patients with MS have been analyzed in relation to (*a*) reaction to exogenous agents such as brain components or viruses, (*b*) cytotoxic or demyelinative effects on nervous tissue, and (*c*) changes in endogenous cell populations.

Response to exogenous agents Many of the reports in this area have been conflicting, with difficulties in assaying cellular responses, presentation of exogenous stimuli (?antigens), and determination of differences between patients with multiple sclerosis and those with other conditions, including normal controls. A major focus has been on responses of lymphocytes to possible brain antigens, such as basic protein, or to viral antigens, particularly those from the measles virus. To this reviewer, the results have been inconclusive in terms of either indicating a response specific to multiple sclerosis or providing information about the mechanism of disease. Attempts have been made with peripheral blood lymphocytes or lymphocytes from CSF to demonstrate cytotoxic effects on tissue explants; these technically difficult studies have not been conclusive. This area of multiple sclerosis research has recently been reviewed (Antel et al 1978).

Two recent approaches to the cellular immunology of multiple sclerosis appear more promising:

1. The observation that T suppressor cells are decreased in both peripheral blood and CSF coincidentally with exacerbation of MS.
2. Fusion studies with lymphocytes.

1. Originally, suppressor cells were assayed by concanavalin A induction (Antel et al (1978). However, a recent report (Reinherz et al 1980) using specific monoclonal antibodies directed against T cells, helper cells, and suppressor cells indicated that most patients had a marked reduction of suppressor cells during an acute attack, with return of this population of T cells during remission. Suppressor cells were not affected in other inflammatory demyelinative diseases such as disseminated encephalomyelitis or the Landry-Guillain-Barre syndrome. In contrast, a similar decrease in suppressor cells has been demonstrated in other possible autoimmune diseases, such as systemic lupus erythematosis, hemolytic anemia, atopic eczema, and inflammatory bowel disease. The precise mechanism of the decrease in suppressor cells is not known, but it may be related to the release of autoantibodies that selectively eliminate the suppressor cell population. The role of inducer and suppressor T lymphocytes in regulation of immune responses has recently been reviewed (Reinherz & Schlossman 1980).

2. The second approach is the fusion of lymphocytes in blood or cerebrospinal fluid with myeloma cell lines to form antibody-generating hybridomas (Sandberg-Wollheim et al 1980). The availability of myeloma cell lines from human may eliminate the interspecies problems that limited earlier attempts with this approach (Croce et al 1980). With this approach it might be possible to determine whether CSF lymphocytes from MS patients generate specific antibodies, particularly during acute exacerbations.

INTERPRETATION Many of the reported changes in peripheral leukocytes have been difficult to reproduce. However, the decrease in suppressor T cells during acute attacks and a return to normal with remission has been reported from a number of laboratories. The relation of this decrease to the mechanism of disease is unclear. Possibilities include common antigens between oligodendroglia and T suppressor cells, a role for T suppressor cells in limiting an autoimmune response during periods of remission, and a nonspecific response similar to the production of most of the oligoclonal immunoglobulin.

An Infectious Etiology to Multiple Sclerosis

The hypothesis of an infectious agent in MS is not new, just difficult to prove. As attempts to demonstrate or culture more conventional agents such as bacteria, spirochetes, or fungi have failed, proponents of this hypothesis have turned to unconventional agents, or conventional agents acting in unconventional ways. This translates in today's terms as the "viral hypothesis of multiple sclerosis." This hypothesis is based on (*a*) the population migration data (reviewed above), (*b*) the immunological responses in

CSF, which are similar to those seen after known chronic panencephalitis of known viral origin, and (*c*) the demonstration in animals that viral infections can produce demyelination as the primary pathology (Johnson 1980).

The strongest argument against the viral hypothesis is that no agent has been reproducibly seen, cultured, rescued, or biochemically identified from brain or other tissue from patients with multiple sclerosis. Even the research strategies used successfully to analyze the viral or transmissible nature of slow viruses, or latent viral infections in the human, such as subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, Jakob-Kreutzfeld disease, or Herpes Simplex infection, have been negative. For example, intracerebral injection of MS brain into primates (Sibley et al 1980), as was used in kuru and Jakob-Kreutzfeld disease (Gajdusek & Gibbs 1975), cocultivating to rescue virus, as was done with progressive multifocal leukoencephalopathy (Weiner et al 1972), and the use of biochemical probes for viral nucleic acids (Dorries & ter Meulen 1980), as has been done in herpes simplex encephalitis (Marsden 1980, Cabrera et al 1980) have all been negative.

Analysis of viral infection in animals suggests several possible mechanisms by which viruses might induce a demyelinative disease (Brooks et al 1979, Fields & Weiner 1981). Direct cytotoxic effect on oligodendroglia occurs with the JHM strain of mouse hepatitis virus and with the JC papavovirus in progressive multifocal leukoencephalopathy. Viral infection might not necessarily be acutely cytotoxic but rather, in a chronic process, might interfere with cellular functions required for maintenance of myelin. In the human disease, this possibility may be difficult to prove if the latent infection is limited to oligodendroglia around plaques. However, if the viral process is more generalized, despite the focal pathology, then current techniques for obtaining oligodendroglia from brain and characterizing their biochemical properties may be applicable. Alternatively, more accessible tissue, such as lymphocytes, might contain the viral genome.

An alternative to direct effect on cellular function by a virus is the possibility that viral infection induces immunologic responses in the host that are damaging to the nervous system. In lymphocytic choriomeningitis infection of mice, the CNS involvement can be prevented by prior immunosuppression of the animals. In addition, the disease can be transferred by immune T cells but not by immune sera (Nathanson et al 1975). In murine encephalomyelitis (Theiler's Virus) there is a two-stage disease, an acute encephalitic involvement of gray matter, and a chronic, delayed involvement of white matter. Immunosuppression enhances the acute phase and abolishes the chronic phase (Lipton & Dalcanto 1976). These observations suggest two possible mechanisms: (*a*) viral infection alters neural cell

membranes to produce an autoimmune type of disease and/or (*b*) there are similar receptors for the virus on both neural cells and peripheral blood lymphocytes. This latter mechanism seems to be operative in recognition of the hemagglutin of reovirus type 3 in which both fatal encephalitis and generation of suppressor cells occur in the course of the disease (Weiner et al 1980a,b).

Repeated attacks of demyelination have not been reported in virally induced demyelinative diseases. However, in visna, a virally-induced demyelinative disease in sheep, the RNA virus persists as a DNA provirus intermediate. The infected animals initially develop a cellular and humoral response to the agent. However, in chronically infected animals, virulent viruses can be recovered by co-cultivation that are not neutralized by the antibodies formed against the initial infectious agent (Narayan et al 1978). This antigenic shift caused by spontaneous mutations of the virus within the host can lead to serial exacerbations of disease with the cumulative effect appearing as progressive neurological disease (Johnson 1980). It is conceivable that a similar mechanism might result in an exacerbating and remitting form of disease.

INTERPRETATION At present there is no hard evidence to substantiate a role for viruses or some other form of transmissible agent in either the onset or recurrent attacks of multiple sclerosis. However, the methods for detecting latent viral infection such as the use of nucleic acid hybridization, monoclonal antibodies, or the use of anti-idiotypic antibodies to determine antigenic determinants on centrally derived immunoglobulin have only recently been applied to multiple sclerosis. Those who subscribe to the viral hypothesis of multiple sclerosis hope these advances will provide positive evidence to substantiate the role of an infectious agent.

Diagnosis of Multiple Sclerosis

The diagnosis of MS rests primarily on the interpretation of the clinical signs and symptoms, with emphasis on the presence of lesions which are spread geographically in the nervous system and spread temporally in terms of exacerbations and remissions.

The symptoms reflect the involvement of white matter. Thus, disruption of tracts presenting as unilateral visual loss (optic nerve), hemiparesis (corticospinal tracts), ataxia (cerebellar outflow systems), internuclear ophthalmoplegia (medial longitudinal fasciculus) are common. In contrast, symptoms relative to neuronal involvement such as seizures, focal deficits in high cortical function, and dementia are uncommon, particularly early in the course of the disease.

The clinical course of the disease is highly variable. Some patients have a fulminant, monotropic disease with inexorable progression. More common is the pattern of exacerbations and remissions followed by a decrease in exacerbations and the appearance of slow progression. Finally, some patients may have one or two episodes and then be symptom-free for many years. These clinical patterns among patients are so different that one wonders if multiple sclerosis is a single disease.

Contrary to the image most people have of multiple sclerosis, the disease carries a reasonable prognosis in most patients. Data from follow-up of patients seen at the Mayo Clinic (Percy et al 1971) and in follow-up of US Army personnel (Kurtzke 1970) indicate that 75% of patients are alive 25 years after the onset of the disease. Of these survivors, 55% are without significant disability. Even these figures may be falsely skewed toward more severely involved patients because of the diagnosis requiring rigid clinical criteria. Thus, early cases and milder cases may be excluded. The true clinical spectrum and prognosis of MS will not be known until a reliable laboratory diagnostic test for multiple sclerosis is available.

LABORATORY AIDS TO DIAGNOSIS Aids to diagnosis are listed in Table 1. The changes in CSF are discussed above. The physiologic tests involving evoked potential recording in the visual, auditory, or somatosensory systems are useful in demonstrating the existence of a second, separate lesion which may be subclinical in its presentation. Evoked responses have also been useful for studying the physiology of recovery from an acute attack, particularly in the visual system (McDonald & Halliday 1977).

INTERPRETATION A laboratory test that is unequivocally diagnostic for multiple sclerosis does not exist. Thus, it is virtually impossible to make the diagnosis early in the course of the disease for many patients; hence the use of such terms as "possible multiple sclerosis," or "probable multiple sclerosis" (this problem in diagnostic classification is well reviewed in Brown et al 1979). Such a diagnostic test is badly needed, not only for individual patients, but also for epidemiological and genetic studies.

Mechanisms of Recovery

A striking feature of multiple sclerosis, particularly early in the course of the disease, is the degree of recovery patients can achieve during remissions. This recovery takes place under circumstances where abnormal physiological responses, such as altered visual evoked potentials, may persist (McDonald & Halliday 1977). Remyelination of plaques is rarely seen, and then only around the edges (Prineas & Connell 1979). Thus, explanations of recovery have centered not on remyelination but on restoration of conduc-

Table I Abnormalities in clinically definite multiple sclerosis

Test	Remarks
Visual evoked responses	Indication of topical disease.
Auditory evoked responses	
Somatosensory evoked responses	
Cerebrospinal fluid:	
IgG concentration	Elevated in approximately 70% of patients. No correlation with disease activity.
IgG Index[a]	Elevated in approximately 70–90% of patients. Possible correlation with disease activity.
Oligoclonal immunoglobulin pattern	Present in 80–90% of patients. No definite correlation with disease activity. Onset of abnormality in early disease not known.
Myelin basic protein	Present during exacerbation. Decrease may correlate with clinical improvement.

[a] CSF IgG index: CSF-IgG/CSF-Albumin/(Serum IgG/Serum albumin.

tion in demyelinated nerve (Bostock & McDonald 1981). The model for this process has been the recovery of conduction in the peripheral nervous system following demyelination with either diphtheria toxin or lysolecithin. In these models, recovery of conduction in persistently demyelinated areas is, in part, related to the spread of sodium channels along the nerve from their normal site at internodes (Ritchie & Rogart 1977, Foster et al 1980, Bostock & McDonald 1981). This type of conduction is slower than normal saltatory conduction (Sears et al 1978)—a finding compatible with the observation in multiple sclerosis of restoration of function despite persistently slowed central conduction (Halliday & McDonald 1977).

INTERPRETATION It is not clear why remyealination does not occur in multiple sclerosis. Possibilities include the following:

1. Adult oligodendroglia do not have the capacity to divide and migrate into the areas of myelin dissolution and loss of oligodendroglia.
2. There is, in the adult nervous system, no pool of immature oligodendroglia capable of migrating to the site of injury and differentiating into reparative, remyelinating cells.
3. Some factors, such as astrocytic processes, altered axonal membrane, or persistance of proteases, interfere with the normal recognition of an unmyelinated axon by a myelinogenic oligodendroglia.

These possibilities are being explored in animal models of demyelination using cell-specific markers.

Therapy

There is no specific therapy for MS. Attempts have been directed at altering immunological responses by immunosuppression, introduction of antigens, and dietary alteration. The unpredictability of the disease, the changing clinical course from one of exacerbations and remissions to one of progression, and the lack of proven laboratory tests to demonstrate clinical activity make therapeutic trials extremely difficult to design and carry out. Guidelines for such clinical trials have recently been published (Brown et al 1979).

Immunosuppression with high doses of steroids or ACTH results in a shortening of the duration of acute attacks (Rose et al 1970). There is no evidence that this form of therapy prevents attacks or alters the progressive form of the disease. The use of other forms of immunosuppression such as azathioprine or cyclophosphamide may reduce the relapse rate, but apparently does not benefit the chronic-progressive forms. Several controlled clinical trials with these agents as well as with antilymphocyte sera are in progress (Bauer 1980).

The use of myelin basic proteins or related copolymers in therapy of multiple sclerosis is based on the observation that these agents can alter the course of the experimental disease, EAE. Preliminary trials with myelin basic protein have not been reported to alter the course of the disease. Dietary therapy has been based on a possible role of unsaturated fatty acids in the disease. Reports of the use of these diets have been inconclusive (Bauer 1980).

Future Directions

It is extremely hazardous to make predictions about a disease with unknown etiology, unknown mechanisms, and unknown therapy. Nevertheless, advances in recent years do suggest some promising leads.

ETIOLOGY The emphasis continues to be on the demonstration of a transmissible agent. Future work in this area may be directed at the demonstration of altered cellular responses induced by a virus. The possible use of peripheral blood leukocytes or lymphocytes in CSF may aid in these investigations, because brain tissue from patients in the acute phases of the disease is rarely available. Thus, studies dependent on demonstration or isolation of an agent from brain are limited by availability of tissue from both patients with multiple sclerosis and appropriate controls.

MECHANISM The immunologic abnormalities in MS have not provided an understanding of the basic mechanism of the disease. However, as mentioned above, the use of anti-idiotypic antibodies as well as more highly

specific monoclonal antibodies may provide significant new information. Such information may be useful not only in understanding the mechanisms of disease, but also in providing a definitive diagnostic test.

THERAPY There are numerous examples in neurology where therapy has been successfully introduced without a clear understanding of the underlying disease process. Such is the case in epilepsy, Parkinson's Disease and manic-depressive illness. A number of new approaches to therapy have been suggested or are under investigation.

Anti-viral agents Despite the absence of evidence implicating a specific virus, attempts at therapy with potential anti-viral agents have been attempted. The most recent trial in this area involves the use of interferon in a controlled clinical trial.

Removal of circulating factors Based on the successful use of plasmapheresis in myasthenia gravis (Dau et al 1977) and chronic relapsing polyneuritis (Server et al 1979), the removal of circulating factors is being evaluated in a controlled trial of therapy during acute attacks of MS.

Modification of properties of demyelinated nerve Assuming that remyelination is not the primary mechanism of recovery in MS, attempts to use agents which might prolong the action potential are being considered. Clinical trials are underway with 4-aminopyridine, a drug which blocks mammalian potassium channels and aids conduction in experimental demyelination (Sherratt et al 1980, Bostock & McDonald 1981).

Alteration of protease actions Attempts to neutralize extracellular effects of such proteases are being considered on the assumption that macrophage-derived neutral proteases selectively attack myelin basic protein.

Immunopharmacologic therapy The current approaches using high doses of steroids, ACTH, or other immunosuppressive drugs have not yielded any striking improvements in the clinical course of the disease, particularly in the chronic progressive form. Similarly, trials with transfer factor have been inconclusive. Agents that might augment suppressor cell functions are under study.

Speculative Overview

Multiple sclerosis continues to be a baffling disease, although new observations continue to be made. Most of those in recent years have been in the immunologic area. What is lacking, however, is a consistent thread that will

tie the epidemiological, genetic, and immunological data together. The most attractive hypothesis continues to be that some form of transmissible agent, acquired early in life by susceptible individuals, alters the host's immune system. In the central nervous system this results in the activation of B cell clones and in the alteration of cellular membranes of oligodendroglia and vascular endothelium. The repeated attacks may be related to a nonspecific immunologic challenge or to antigenic shifts of the latent transmissible agent. So much for theory. How to prove this or other possible hypotheses, and how to translate that proof into an alteration of the disease remains the challenge.

Acknowledgments

Personal work referred to in this review was supported by grants from the National Multiple Sclerosis Society and from the National Institute of Neurological Diseases, Communicative Disorders, and Strokes.

Literature Cited

Abramsky, O., Lisak, R. P., Silberberg, D. H., Pleasure, D. E. 1977. Antibodies to oligondendroglia in patients with multiple sclerosis. *N. Engl. J. Med.* 297: 1207–11

Adams, C. W. M. 1977. Pathology of multiple sclerosis: Progression of the lesion. *Br. Med. Bull.* 33:15–20

Allen, I. U. 1980. A histological and histochemical study of the macroscopically normal white matter in multiple sclerosis. In *Progress in Multiple Sclerosis Research*, ed. H. J. Bauer, S. Poser, G. Ritter, pp. 340–47. Berlin/Heidelberg/New York: Springer. 677 pp.

Alter, M. 1980. The geographic distribution of multiple sclerosis: New concepts. See Allen 1980, pp. 495–502

Alter, M., Kahana, E., Loewenson, R. 1978. Migration and risk of multiple sclerosis. *Neurology* 28:1089–98

Antel, J. P., Arnason, B. G. W., Medof, M. E. 1978. Suppressor cell function in multiple sclerosis: Correlation with clinical disease activity. *Ann. Neurol.* 5:338–42

Antel, J. P., Richman, D. P., Medof, M. E., Arnason, B. G. W. 1978. Lymphocyte function and the role of regulator cells in multiple sclerosis. *Neurology* 28(2): 106–10

Baird, L. G., Tachovsky, T. G., Sandberg-Wollheim, M., Kaprowski, H., Nisonoff, A. 1980. Identification of a unique idiotype in cerebrospinal fluid and serum of a patient with multiple sclerosis. *J. Immunol.* 124:2324–28

Bauer, H. J. 1980. Recent trends in the therapy of multiple sclerosis. See Allen 1980, pp. 653–56

Bloom, B. R. 1980. Immunological changes in multiple sclerosis. *Nature* 287: 275–76

Bloom, B. R., Ju, G., Brosnan, C., Cammer, W., Norton, W. 1978. Notes on the pathogenesis of multiple sclerosis. *Neurology* 28(2):93–101

Bornstein, M. B. 1963. A tissue culture approach to demyelinative disorders. *Natl. Cancer Inst. Monogr.* 11:197–214

Bornstein, M. B., Crain, S. M. 1965. Functional studies of cultured brain tissues as related to demyelinative disorders. *Science* 148:1242–44

Bostock, H., McDonald, W. I. 1981. Recovery of function after demyelination. In *Neuronal-Glial Cell Interrelationships*, ed. T. A. Sears. Dahlen Konferenzen. Weinheim/Deerfield Beach/Basel: Verlag Chemie. In press

Brooks, B. R., Jubelt, B., Swarz, J. R., Johnson, R. T. 1979. Slow viral infections. *Ann. Rev. Neurosci.* 2:309–40

Brosnan, C. F., Cammer, W., Norton, W. T., Bloom, B. R. 1980. The role of activated macrophages in inflammatory demyelinating diseases. *4th Int. Cong. Immunol.* 18.6.05.

Brown, J. R., Beebe, G. W., Kurtzke, J. F., Loewenson, R. B., Silberberg, D. H., Tourtelotte, W. W. 1979. The design of

clinical studies to assess therapeutic efficacy in multiple sclerosis. *Neurology* 29(2):1–23

Cabrera, C. V., Wohlenberg, C., Openshaw, H., Ray-Mendez, M., Puga, A., Notkins, A. L. 1980. Herpes simplex virus DNA sequences in the CNS of latently infected mice. *Nature* 188:288–90

Carson, J. H., Barbarese, E., Braun, P. E., McPherson, T. A. 1978. Components in multiple sclerosis cerebrospinal fluid that are detected by radioimmunoassay for myelin basic protein. *Proc. Natl. Acad. Sci. USA* 75:1976–78

Cohen, S. R., Brooks, B. R., Herndon, R. M., McKhann, G. M. 1980. A diagnostic index of active demyelination—myelin basic protein in cerebrospinal fluid. *Ann. Neurol.* 8:25–31

Cohen, S. R., Herndon, R. M., McKhann, G. M. 1976. Radioimmunoassay of myelin basic protein in spinal fluid: An index of active demyelination. *N. Engl. J. Med.* 295:1455–57

Croce, C. M., Linnenbach, A., Hall, W., Steplewski, Z., Koprowski, H. 1980. Production of human hybridomas secreting antibodies to measles virus. *Nature* 288:488–89

Dau, P. C., Lindstrom, J. M., Cassel, C. K., Denys, E. H., Sher, E. F., Spitler, L. E. 1977. Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. *N. Engl. J. Med.* 297:1134–40

Dean, G. 1980. Multiple sclerosis among immigrants to Britain and in the Islands of Scilly and Malta. See Allen 1980, pp. 519–24

Dean, G., Brady, R., McLoughlin, H., Elian, M., Adelstein, A. M. 1977. Motor neurone disease and multiple sclerosis among immigrants to Britain. *Br. J. Prev. Soc. Med.* 31:141–46

Dorries, K., ter Meulen, V. 1980. Search for viral nucleic acids in multiple sclerosis brain. See Allen 1980, pp. 47–52

Ebers, G. C., Zabriske, J. B., Kunkel, H. G. 1979. Oligoclonal immunoglobulins in subacute sclerosing panencephalitis and multiple sclerosis: A study of idiotypic determinants. *Clin. Exp. Immunol.* 35:67–75

Fewster, M. E., Blackstone, S. 1975. In vitro study of bovine oligodendroglia. *Neurobiology* 5:316–28

Fields, B. N., Weiner, H. L. 1981. Mechanism of viral injury to the nervous system. See Bostock & McDonald 1981, in press

Foster, R. E., Whalen, C. C., Waxman, S. G. 1980. Reorganisation of the axon membrane in demyelinated peripheral nerve fibers: Morphological evidence. *Science* 210:661–63

Gajdusek, D. C., Gibbs, C. J. Jr. 1975. Slow virus infections of the nervous system and the laboratories of slow, latent and temperate virus infections. In *The Nervous System*, ed. D. B. Tower, 2:113–35. New York: Raven. 437 pp.

Grundke-Iqbal, I., Bornstein, M. B. 1979. Multiple sclerosis: Immunological studies on the demyelinating serum factor. *Brain Res.* 160:489–503

Halliday, A. M., McDonald, W. I. 1977. Pathophysiology of demyelinating disease. *Br. Med. Bull.* 33:21–27

Itoyama, Y., Sternberger, H. H., Webster, H. de F., Quarles, R. H., Cohen, S. R., Richardson, E. P. 1980. Immunocytochemical observation on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. *Ann. Neurol.* 7:167–77

Johnson, R. T. 1980. Selective vulnerability of neural cells to viral infections. *Brain* 103:447–72

Johnson, R. T., Katzmann, R., McGeer, E., Price, D., Shooter, E. M., Silberberg, D. 1979. Report of the panel on inflammatory, demyelinating and degenerative diseases. NIH Publ. No. 79–1916. Washington DC: US Dept. Health, Educ., Welfare

Kennedy, P. G. E., Lisak, R. P. 1979. A search for antibodies against glial cells in the serum and cerebrospinal fluid of patients with multiple sclerosis and Guillain-Barre syndrome. *J. Neurol. Sci.* 44:125–33

Kennedy, P. G. E., Lisak, R. P. 1981. Do patients with demyelinating diseases have antibodies against human glial cells in their sera? *J. Neurol. Neurosurg. Psychiatr.* In press

Kennedy, P. G. E., Lisak, R. P., Raff, M. C. 1980. Cell type specific markers for human glial and neuronal cells in culture. *Lab. Invest.* 43:342–51

Kohler, G., Milstein, C. 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511–19

Kurland, L. T. 1970. The epidemiological characteristics of multiple sclerosis. In *Handbook of Clinical Neurology*, ed. P. J. Vinken, G. W. Bruyn, 9:63–84. Amsterdam: North-Holland. 706 pp.

Kurtzke, J. F. 1970. Clinical manifestations of multiple sclerosis. See Kurtzke 1970, pp. 161–216

Kurtzke, J. F. 1975. A reassessment of the distribution of multiple sclerosis. *Acta Neurol. Scand.* 51:110–57

Kurtzke, J. F. 1980. Clustering and epidemic occurrence of multiple sclerosis. See Allen 1980, pp. 519–24

Laterre, E. C. 1966. Les gammaglobulins du liquide cephalo-rachidien dans la sclerose en plaque. *Acta Neurol. Belg.* 66:305–18

Link, H. 1973. Comparison of electrophoresis of agar gel and agarose gel in the evaluation of fluid gamma-globulin abnormalities in cerebrospinal fluid and serum in multiple sclerosis. *Clin. Chim. Acta* 46:383–89

Lipton, H. L., Dalcanto, M. C. 1976. Theiler's virus-induced demyelination: Prevention by immunosuppression. *Science* 192:62–64

Marsden, H. 1980. Herpes simplex virus in latent infection. *Nature* 288:212–13

Mattson, D. H., Roos, R. P., Arnason, B. G. W. 1980. Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. *Nature* 287:335–37

McDonald, W. I., Halliday, A. M. 1977. Diagnosis and classification of multiple sclerosis. *Br. Med. Bull.* 33:4–9

Morell, P. 1977. *Myelin.* New York/London: Plenum. 531 pp.

Nagelkerken, L. M., Aalberse, R. C., van Walbeck, H. K., Out, T. A. 1980. Preparation of antisera directed against the idiotype(s) of immunoglobulin G from the cerebrospinal fluid of patients with multiple sclerosis. *J. Immunol.* 125: 384–89

Narayan, O., Griffin, D. E., Clements, J. E. 1978. Virus mutation during "slow infection." Temporal development and characterisation of mutants of visna virus recovered from sheep. *J. Gen. Virol.* 41:343–52

Nathanson, N., Monjan, A. A., Panitch, H. S., Johnson, E. D., Petursson, G., Cole, G. A. 1975. Virus-induced cell-mediated immunopathological disease. In *Viral Immunology and Immunopathology*, ed. A. Notkins, pp. 357–91. New York: Academic

Norby, E. 1978. Viral antibodies in multiple sclerosis. *Prog. Med. Virol.* 24:1–39

Oger, J. J. F., Arnason, B. G. W. 1980. HLA patterns in multiple sclerosis. See Allen 1980, pp. 460–64

Palo, J. 1978. *Myelination and Demyelination.* New York/London: Plenum. 651 pp.

Paterson, P. Y., Day, E. D., Varitek, V. A., Peterson, D. J., Whitacre, C. C., Berenberg, R. A., Harter, D. H. 1980. Circulating myelin basic protein (MBP) fragments: Role in neuroimmunologic diseases. *4th Int. Cong. Immunol.* 18.-6.22

Paterson, P. Y., Day, E. D., Whitacre, C. C., Berenberg, R. A., Harter, D. H. 1981. Endogenous myelin basic protein-serum factors (MBP-SFs) and anti-MBP antibodies in humans. Occurence in sera of clinically well subjects and patients with multiple sclerosis. *J. Neurol. Sci.* In press

Percy, A. K., Nobrega, F. T., Okazaki, H., Glattre, E., Kurland, L. T. 1971. Multiple sclerosis in Rochester, Minn. *Arch. Neurol.* 25:105–11

Poduslo, S. E., Miller, K., McKhann, G. M. 1978. Metabolic properties of maintained oligodendroglia. *J. Biol. Chem.* 253:1592–97

Poduslo, S. E., Norton, W. T. 1972. Isolation and some chemical properties of oligodendroglia from calf brain. *J. Neurochem.* 19:727–36

Prineas, J. W., Connell, F. 1978. The fine structure of chronically active multiple sclerosis plaques. *Neurology* 28(2): 68–75

Prineas, J. W., Connell, F. 1979. Remyelination in multiple sclerosis. *Ann. Neurol.* 5:22–31

Raff, M. C., Fields, K. L., Hakomori, S.-I., Mirsky, R., Pruss, R. M., Winter, J. 1979. Cell-type specific markers for distinguishing and studying neurones and the major class of glial cells in culture. *Brain Res.* 174:283–308

Raff, M. C., Mirsky, R., Fields, K. L., Lisak, R. P., Dorfman, S. H., Silberberg, D. H., Gregson, N. A., Leibowitz, S., Kennedy, M. 1978. Galactocerebroside is a specific cell surface antigenic marker for oligodendrocytes in culture. *Nature* 274:813–16

Raine, C. S., Traugott, H. U., Stone, S. H. 1980. Applications of chronic relapsing experimental allergic encephalomyelitis to the study of multiple sclerosis. See Allen 1980, pp. 3–10

Reinherz, E. L., Schlossman, S. F. 1980. Regulation of the immune response—inducer and suppressor T lymphocyte subsets in human beings. *N. Engl. J. Med.* 303:370–73

Reinherz, E. L., Weiner, H. L., Hauser, S. L., Cohen, J. A., Distaso, J. A., Schlossman, S. F. 1980. Loss of suppressor T cells in active multiple sclerosis. Analysis with monoclonal antibodies. *N. Engl. J. Med.* 303:125–29

Richards, P. T., Cuzner, M. L. 1978. Proteolytic activity in cerebrospinal fluid. *Adv. Exp. Med. Biol.* 100:521–27

Ritchie, J. M., Rogart, R. B. 1977. The density of sodium channels in mammalian myelinated nerve fibres and the nature of the axonal membrane under the myelin sheath. *Proc. Natl. Acad. Sci. USA* 74:211–15

Rose, H. S., Kuzma, J. W., Kurtzke, J. F., Sibley, W. A., Tourtelotte, W. W. 1970. Cooperative study in the evaluation of therapy in multiple sclerosis: ACTH as placebo in acute exacerbations. Final report. *Neurology* 20(2):1–59

Sandberg-Wollheim, M., Shander, M., Croce, C. M., Kaprowski, H. 1980. Production of interspecies hybridomas between CSF or blood lymphocytes from patients with neurological diseases and mouse myeloma cells. See Allen 1980

Schachner, M., Commer, L., Lagenaur, C., Schnitzer, J. 1981. Developmental expression of antigenic markers in glial subclasses. See Bostock & McDonald 1981, in press

Schauf, C. L., Davis, F. A. 1978. The occurrence, specificity and the role of neuroelectric blocking factors in multiple sclerosis. *Neurology* 28(2):34–39

Sears, T. A., Bostock, H., Sherratt, R. M. 1978. The pathophysiology of demyelination and its implication for the symptomatic treatment of multiple sclerosis. *Neurology* 28(2):21–26

Seil, F. J. 1977. Tissue culture studies in demyelinating diseases: A critical review. *Ann. Neurol.* 2:345–55

Server, A. C., Lefkowith, J., Braine, H., McKhann, G. M. 1979. Treatment of chronic relapsing inflammatory polyradiculneuropathy by plasma exchange. *Ann. Neurol.* 6:258–61

Sherratt, R. M., Bostock, H., Sears, T. A. 1980. Effects of 4-aminopyridine on normal and demyelinated mammalian nerve fibres. *Nature* 283:570–72

Shorr, J., Roström, B., Link, H. 1981. Antibodies to viral and non-viral antigens in subacute sclerosing panencephalitis and multiple sclerosis demonstrated by thin-layer polyacrylamide gel isoelectric focusing, antigen immunofixation and autoradiography. *J. Neurol. Sci.* 49:99–108

Sibley, W. A., Kalter, S. S., Laguna, J. F. 1980. Attempts to transmit multiple sclerosis to newborn and germ-free non-human primates: A ten-year interim report. See Allen 1980, pp. 80–85

Snyder, D. S., Raine, C. S., Faroog, M., Norton, W. T. 1980. The bulk isolation of oligodendroglia from whole rat forebrain: A new procedure using physiologic media. *J. Neurochem.* 34: 1614–21

Szuchet, S., Stefansson, K., Wallmann, R. L., Dawson, G., Arnason, B. G. W. 1980. Maintenance of isolated oligodendrocytes in long-term culture. *Brain Res.* 200:151–64

Tachovsky, T. G., Baird, L. G. 1980. Unique idiotypes in CSF of MS patients. *4th Int. Congr. Immunol.* 18.6.28

Thompson, E. J. 1977. Laboratory diagnosis of multiple sclerosis: Immunological and biochemical aspects. *Br. Med. Bull.* 33:28–33

Traugott, U., Snyder, D. S., Raine, C. S. 1979. Oligodendrocyte staining by multiple sclerosis serum is non-specific. *Ann. Neurol.* 6:13–20

Trotter, J. L., Huss, B., Blank, W. P., O'Connell, K., Hagen, S., Shearer, W. T., Agrawal, H. C. 1978. Myelin basic protein and CSF in normal and pathological brains. *Trans. Am. Soc. Neurochem.* 9:59

Vandvik, B., Norby, E. 1980. Viral antibody responses in the central nervous system of patients with multiple sclerosis. See Allen 1980

Weiner, H. L., Ault, K. A., Fields, B. N. 1980a. Interaction of reovirus with cell surface receptors. I. Murine and human lymphocytes have a receptor for the hemagglutination of reovirus type 3. *J. Immunol.* 124:2143–48

Weiner, H. L., Powers, M. L., Fields, B. N. 1980b. Reovirus virulence and central nervous system cell tropism: Absolute linkage to the viral hemagglutination. *J. Inf. Dis.* 141:609–16

Weiner, L. P., Hendon, R. M., Narayan, O., Johnson, R. T. 1972. Further studies of an SV40-like virus isolated from human brain. *J. Virol.* 10:147–52

Whitaker, J. N. 1977. Myelin encephalitogenic protein fragments in cerebrospinal fluid of persons with multiple sclerosis. *Neurology* 27:911–20

Whitaker, J. N., Bashir, R. M., Jen Chou, C-H., Kibler, R. F. 1980. Antigenic features of myelin besic protein-like material in cerebrospinal fluid. *J. Immunol.* 124:1148–53

Wisniewski, H. M., Lassmann, H. 1980. Chronic relapsing EAE: Its application to study of human inflammatory demyelinating diseases. See Allen 1980

EXHIBIT G

Autoimmune Disease and the Nervous System
Biochemical, Molecular, and Clinical Update

Moderator
JEAN E. MERRILL, PhD

Discussants
MICHAEL C. GRAVES, MD, *and* DONALD G. MULDER, MD, *Los Angeles, California*

This is an edited summary of an Interdepartmental Conference arranged by the Department of Medicine of the UCLA School of Medicine, Los Angeles. The Director of Conferences is Anthony T. Campagnoni, PhD, Professor of Medicine.

Autoimmunity in the central and peripheral nervous system can manifest as the result of cellular or humoral immune responses to autoantigens. There is evidence that multiple sclerosis is a cell-mediated autoimmune disease of the central nervous system in which both myelin and the cell that produces the myelin are destroyed. Diseases such as acute inflammatory demyelinating polyneuropathy (also called Guillain-Barré syndrome) and myasthenia gravis are considered antibody-mediated diseases of the peripheral nervous system and neuromuscular junctions, respectively. We review these diseases and explore mechanisms of immune-mediated destruction of these nervous system components. We specifically focus on one effective therapy aimed at countering the immune attack, that of thymectomy in patients with myasthenia gravis.

(Merrill JE, Graves MC, Mulder DG: Autoimmune disease and the nervous system—Biochemical, molecular, and clinical update. West J Med 1992 Jun; 156:639-646)

JEAN E. MERRILL, PhD*: Multiple sclerosis is a demyelinating disease of the white matter of the central nervous system (CNS) in which myelin and the myelin-producing macroglia cell, the oligodendrocyte, are destroyed. Axons are usually spared. In response to the damage, macroglial astrocytes in the central nervous system undergo proliferation and hypertrophy, which is called astrogliosis.[1] Multiple sclerosis is thought to be an autoimmune disease for reasons that will be discussed.† Both blood-borne inflammatory cells and endogenous glial cells (astrocytes as well as brain macrophages called microglia) have been implicated in the disease process.[2-4] Several abnormalities have been documented in subsets and functions of lymphocyte subpopulations[2,5-7] and will not be discussed here. Multiple sclerosis is diagnosed by neurologic examination, laboratory studies of immunoglobulins in cerebrospinal fluid (CSF), and, more recently, noninvasive magnetic resonance imaging.[2-4]

The Trimolecular Complex

The three components of the trimolecular complex that seem to be most critical for autoimmune disease, or reactivity to self-antigens, are the major histocompatibility complex, class II molecules (MHC or HLA-DR) expressed on various antigen-presenting cells, the antigenic peptide, and the T-cell receptor on $CD4^+$ T-cell subsets.[8-10] In the animal model for multiple sclerosis, experimental allergic encephalomyelitis (EAE), specific encephalitogenic peptides from the myelin constituent myelin basic protein, when presented on restricted MHC-II molecules in susceptible murine strains, induce highly restricted T-cell receptor variable region genes on $CD4^+$ cells.[11-14] In multiple sclerosis, we have less information regarding these three molecules. The MHC-II molecules DR2 and DRw2 are overrepresented in patients with multiple sclerosis.[2,15] The MHC-II molecules in multiple sclerosis are expressed on endothelial cells of the blood-brain barrier, on microglia and astrocytes, and on inflammatory macrophages.[16-19] Any of these cells could behave as antigen-presenting cells to present self-antigen to $CD4^+$ T cells.

The antigen that induces or perpetuates, or both, the chronicity of the disease is unknown. Epidemiologic and demographic studies suggest an exogenous infectious agent, such as a virus, as a candidate immunogen. Tolerance to self would then be broken either by viral alteration of a normal self-antigen through infection of a brain cell or through a cross reactivity called molecular mimicry in which the viral antigen resembles a normal brain antigen.[20] In addition, an imbalance in the immune system T-cell subsets in patients with multiple sclerosis is thought to play a part in inducing disease. A functional or numerical increase in $CD4^+$ helper-inducer T cells (cells that mediate delayed-type hypersensitivity) and a decrease in $CD8^+$ suppressor-cytotoxic T cells have been shown to occur in patients with multiple sclerosis. $CD4^+$ cells exposed to the correct autoreactive or cross-reactive antigen, presented by restricted MHC-II molecules, would then give rise to autoimmunity.[2,13,15]

*Professor, Department of Neurology, UCLA School of Medicine.

†See also "Autoimmune Disease and the Nervous System," by L. Steinman, MD, pages 664-666 of this issue.

This work was supported in part by National Institutes of Health grant NS26983 and the Conrad Hilton Foundation and Psychoneuroimmunology Task Force, University of California, Los Angeles.

Reprint requests to Jean E. Merrill, PhD, Department of Neurology, UCLA School of Medicine, University of California, Los Angeles, CA 90024.

ABBREVIATIONS USED IN TEXT

AChR = acetylcholine receptor
CMV = cytomegalovirus
CNS = central nervous system
CSF = cerebrospinal fluid
EAE = experimental allergic encephalomyelitis
IFN-γ = interferon gamma
IL = interleukin
MHC = major histocompatibility complex
TGF = transforming growth factor
TNF = tumor necrosis factor

T Lymphocytes and Interleukin 2

Activated T cells from peripheral blood, whether they are specific to brain antigens or not, can and do cross into the CNS through an intact blood-brain barrier.[21] The mechanisms by which this occurs are not known. If these activated T cells recognize antigens in the brain, they can cause damage in the CNS. Measurement and detection using various techniques have shown that the $CD4^+$ T cells predominate over the $CD8^+$ T cells in the lesion, spinal fluid, and blood of patients with multiple sclerosis.[17,22-27] The $CD4^+$ cells are activated in that they express MHC-II, a T-cell differentiation marker (TA-1), and interleukin (IL)-2 receptors (TAC) and produce cytokines.[17,24-26] Some of these cells respond to brain antigens.[22,26,28] In addition, although there may be some restriction in gene usage in the T-cell receptor in multiple sclerosis,[23,29,30] it is not as limited in heterogeneity as is found in the EAE model.[7-9]

Elevated levels of IL-2 and IL-2R have been detected in CSF and serum of patients with multiple sclerosis,[31-38] as well as in tissue.[17] Serum levels of IL-2 correlate inversely with disability and directly with a poor prognosis.[34] In patients with chronic disease, the magnitude of the initial IL-2 levels varies inversely with the duration of disease and predicts subsequent worsening.[37] An association of T cells capable of producing and responding to IL-2 and disease duration, worsening, or both, implies that interleukin 2 is expanding encephalitogenic clones in the periphery and locally in the CNS. Thus, although this is not unique to patients with multiple sclerosis in that other autoimmune and inflammatory diseases show similar findings, it is indicative of an activated response of the immune system that may correlate with the fluctuations in symptoms.

Interferon Gamma and Major Histocompatibility Complex Class II Molecules

Interferon gamma (IFN-γ) can induce or increase the expression of MHC-II molecules on T cells, macrophages, microglia, and astrocytes either directly or indirectly through the induction of tumor necrosis factor (TNF) α.[39-43] Interferon gamma and MHC-II, although not expressed in normal brain, are expressed in multiple sclerosis plaques, and the staining for these is associated with macrophages and astrocytes in lesions.[17,18,44] In recent studies, T cells of patients with multiple sclerosis stimulated in vitro were shown to produce increased amounts of IFN-γ compared with T cells of control subjects.[45-47] Interferon gamma is also detectable in the CSF of patients with multiple sclerosis. Activation of the immune system in patients with multiple sclerosis by clinical treatment with IFN-γ leads to exacerbations and an increase in circulating MHC-II-positive macrophages.[48,49]

Macrophages and Cytokines

Prostaglandin E, interleukin 1, and tumor necrosis factor-α. Macrophages in blood, CSF, and brain of patients with multiple sclerosis are activated. They have been shown to actively cap immunoglobulin on myelinated nerve fibers at plaque margins[50] and to produce prostaglandin E and IL-1 at the blood-brain barrier and in the lesion.[17] Prostaglandin E levels are elevated in the CSF of patients with multiple sclerosis and in vitro in cultures of stimulated blood macrophages.[51,52] In multiple sclerosis, macrophages show increased oxygen burst in vitro and the CSF contains autooxidative products of degraded membrane phospholipids, both of which are consistent with lipid peroxidation and myelin destruction.[53,55] Interleukin 1 and TNFα coinduce each other and stimulate the production of prostaglandin E,[56-59] which regulates temperature and fever.[56-58] Because patients with multiple sclerosis show a worsening in neurologic status during febrile episodes,[60] IL-1, TNFα, and prostaglandin E may contribute to the disease process through fever induction. In vitro studies in multiple sclerosis have confirmed that production by macrophages of IL-1[61-63] and TNFα[46] is elevated. Both macrophages and glial cells are associated with these cytokines in situ in the lesions.[64]

Examination of serum and CSF specimens for such cytokines as IL-1, IL-6, and TNF has led to less clear findings of undetectable, elevated, or normal levels of cytokines compared with levels in fluids from control subjects.[63,65-68] Some of the confounding problems in measuring cytokines in biologic fluids from patients and correlating the results with their disease are the short half-life of these proteins, the concomitant production and presence of inhibitors, and the possible induction in vivo by bacterial infections unrelated to the disease process of interest.

TABLE 1.—*Putative Roles for Cytokines in Demyelinating Disease*

Cytokine	*Possible Function in Multiple Sclerosis*
Interleukin (IL)-1 + TNFα	Astrogliosis Microgliosis Oligodendrocyte cell death Induction of fever and decreased electric conductivity along demyelinated fiber
Interferon gamma	Increased MHC-II on macrophages, T cells, microglia, and astrocytes Increased cytotoxicity by macrophages and microglia Increased permeability of blood-brain barrier
Transforming growth factor β	Decreased function of IL-1 and TNFα Inhibition of function of activated macrophages Inhibition of T-cell response to IL-2

TNFα = tumor necrosis factor-α

Role of interleukin 1 and tumor necrosis factor-α in lesion formation. Astrocytes produce IL-1 and TNF and respond to these cytokines by proliferation and differentiation.[69-75] It is not clear whether the response is direct or indirect through the induction of other factors or cytokines by IL-1 and TNFα.[74,75] Nevertheless, the effects of these cytokines, direct or indirect, could lead to astrogliosis and plaque formation in multiple sclerosis. The damage to myelin and the oligodendrocyte has long been thought to be the result of activated inflammatory blood macrophages and endogenous microglia.[1,15,19,76,77] Both TNFα[78,79] and IL-1[75] have been implicated. It is not certain, however, whether microglia- or

macrophage-bound membrane cytokines or their soluble counterparts—as in the case of TNFα—are responsible for the death of oligodendrocytes.[76] Of interest, transforming growth factor (TGF) β, a cytokine that inhibits the biologic function of IL-1 and TNFα,[80] also inhibits microglial cytotoxicity of oligodendrocytes.[76] The use of TGFβ effectively treats and prevents EAE in vivo in animals.[81] The putative roles for cytokines in demyelinating disease are shown in Table 1.

Therapy for Multiple Sclerosis

Immunointervention in multiple sclerosis has been generally targeted at various elements of the immune response and particularly at components of the trimolecular structure. Earlier therapeutic protocols using immunosuppressive drugs resulted in modest improvement but proved to be somewhat disappointing because of their nonspecificity, inefficiency, or toxicity.[82-85] Although active vaccination to the T-cell receptor on autoreactive T cells in the EAE model has proved efficient at preventing disease,[86] such preliminary studies have just begun in multiple sclerosis and may prove difficult.[87] The passive administration of antibody to block the T-cell receptor MHC-II molecules and ancillary T-cell molecules like CD4 and CD3 or cytokine receptors has been contemplated[88] and actually carried out in some patients.[89]

The use of natural antagonists to cytokines has proved to be a promising approach. The use of TGFβ might inhibit IL-1 and TNFα production in patients with multiple sclerosis.[76,80,81] Interferons alfa and beta, natural antagonists of the immunologically mediated actions of IFN-γ, have ameliorating effects in vivo in multiple sclerosis.[90-92] The mechanisms of this beneficial effect are thought to be the following: the inhibition of IFN-γ-induced MHC-II by IFN-β,[93] which would inhibit antigen presentations by antigen-presenting cells; a reversal of nonspecific suppression in multiple sclerosis[94]; and the inhibition of IFN-γ secretion by T cells in multiple sclerosis.[95]

Antibody-Mediated Neurologic Diseases

MICHAEL C. GRAVES, MD*: The motor unit is the final common pathway for the activation and control of muscular movement. A typical motor unit consists of an anterior horn cell body, its myelinated axon, and the collection of muscle cells innervated by terminal branches of the axon.

Table 2 summarizes some diseases with symptoms caused by dysfunction at various parts of the motor unit. In myasthenia gravis and myasthenic syndrome, antibodies directed at specific ion channels are the cause of the disease. The other conditions listed in Table 2 have defects located at other specific points along the motor unit, and they also have at least some characteristics common to myasthenia and other autoimmune diseases.

The following findings are common for the better characterized autoimmune diseases such as myasthenia gravis and may offer clues to the pathogenesis of the other conditions:

- An antibody is directed at a specific target, which can explain the pathophysiology of the disease;
- The antibody is present in patients but not controls;
- The disease can be transferred to an experimental host using antibody;
- Symptoms can be lessened with immunosuppressive treatment or plasmapheresis;
- The diseases are commonly associated with other autoimmune diseases; and
- The diseases may be associated with neoplasms.

Two or more of these characteristics are present for all of the diseases listed in Table 2. Specific antibody directed at the presynaptic and postsynaptic sides of the myoneural junction explain the pathophysiology of myasthenic syndrome and myasthenia gravis, respectively; these antibodies can also transfer the disease to laboratory animals. Antibodies directed at myelin are found in demyelinating inflammatory polyneuropathies, and antibodies directed at neuronal gangliosides are present in axonal neuropathies. The latter are sometimes present in other conditions, however, and experiments for the passive transfer of the neuropathy with serum have not yet been done. Associations with other autoimmune diseases are known for myasthenia and for demyelinating neuropathies. Myasthenia gravis, myasthenic syndrome, and the "stiff-man" syndrome are associated with tumors. All of these diseases have been treated with corticosteroids, plasmapheresis, or other immunosuppressive treatments with at least some degree of success.

TABLE 2.—*Motor Unit Dysfunction in Autoimmune Neuromuscular Diseases*

Location of Dysfunction	*Disease*
Inhibitory interneurons	"Stiff-man" syndrome
Peripheral nerve axons	Axonal neuropathies
Peripheral nerve myelin	Demyelinating neuropathies
Myoneural junction	
Presynaptic	Myasthenic syndrome
Postsynaptic	Myasthenia gravis

'Stiff-Man' Syndrome

The stiff-man syndrome is a rare condition of continuous muscle activity at rest.[96] Some patients have stiffness of one or more limbs but continue to function. In more severely affected patients, the condition is similar to tetanus or strychnine poisoning, with incapacitating rigidity and muscle spasms, respiratory failure, fever, elevations of serum creatine kinase levels, and a remarkable response to benzodiazepine medications.[97,98] These findings suggest dysfunction of an important inhibitory synapse on the motor neuron. The presence of antibodies to glutamic acid decarboxylase, an enzyme that produces the inhibitory neurotransmitter γ-aminobutyric acid, has been reported and seems to explain the motor unit activity that characterizes the syndrome,[99] but the antibodies have not been found by other investigators.[100] More recent reports of autoantibodies reactive with neurons containing γ-aminobutyric acid, if confirmed, would certainly explain the pathophysiology of the syndrome.[101] Patients often have pleocytosis and elevated levels of IgG in cerebrospinal fluid. The association with diabetes may relate to the presence of antibodies to pancreatic beta cells.[101] The association with neoplasms may also be an indirect indication of autoimmunity. Severely affected patients have benefited from treatment with corticosteroids or plasmapheresis.[102]

*Associate Professor, Department of Neurology, UCLA School of Medicine.

Motor Neuron Diseases

Motor neuron diseases are conditions characterized by a loss of motor neurons with resultant muscle weakness and atrophy. In typical cases of amyotrophic lateral sclerosis, both the corticospinal and the anterior horn cells degenerate, causing a mixture of upper and lower motor neuron signs.[103] Bulbar involvement may lead to dysphagia and dysarthria. The presence of antibodies directed at neuronal gangliosides, and occasionally of IgM paraproteins, has renewed interest in an autoimmune cause for amyotrophic lateral sclerosis. Drachman and Kuncl coined the term "unconventional autoimmune disease" to emphasize that any autoimmune cause for amyotrophic lateral sclerosis will be unique and different from the causes of conventional autoimmune diseases such as myasthenia.[104] A useful immunosuppressive treatment for most cases of amyotrophic lateral sclerosis has not yet evolved. Occasional anecdotal and sometimes published atypical cases of motor neuron diseases have shown favorable response to immunosuppression, however.

Patients with the following characteristics may represent a subtype of motor neuron disease: lack of upper motor neuron involvement, lack of bulbar involvement, conduction block of motor axons on nerve conduction studies, and high levels of antibodies directed at the GM-1 ganglioside or at other related glycolipids.[105,106] Some of these patients have IgM paraproteins directed at the ganglioside antigens. Immunosuppressive treatment has been reported to benefit some patients with these characteristics. In some cases, initial treatment with corticosteroids, azathioprine, and apheresis has not been helpful, but benefit has been reported with the use of cyclophosphamide.[107] The IgM antibodies directed at gangliosides seem to be difficult to suppress with treatments that have been successful in myasthenia and other autoimmune disorders.[107,108]

Acute Inflammatory Demyelinating Polyneuropathy

Acute inflammatory demyelinating polyneuropathy, also known as the Guillain-Barré syndrome, is a paralytic condition caused by immune-mediated demyelination of peripheral nerve.[109] Acute inflammatory demyelinating polyneuropathy may be associated with antecedent viral infection. Chronic inflammatory demyelinating polyneuropathy follows a chronic worsening or a relapsing remitting clinical course.[110] Antibodies to myelin and to specific carbohydrate structures of glycolipids and glycoproteins have been reported in patients with these diseases.[109] These antibodies are generally IgM. Occasionally patients with monoclonal gammopathies directed at similar determinants have a similar demyelinating polyneuropathy.[111,112] Experimental microinjection of patients' serum can demyelinate nerves of experimental animals.[113] Treatment with plasmapheresis is effective in both acute and chronic inflammatory demyelinating polyneuropathies, and corticosteroids or immune suppression is effective treatment of the chronic disorder.

Myasthenia Gravis

Myasthenia gravis is a disease of weakness and fatigue, frequently affecting swallowing, speech, eye movements, and facial muscles.[114] Antibodies directed at acetylcholine receptor (AChR) are present in most patients, and evidence for the passive transfer of transient myasthenia to newborns of myasthenic mothers has long been appreciated. Passive transfer with patients' IgG or with monoclonal antibodies to the receptor is further evidence for an autoimmune antibody-mediated mechanism.[115] The effect of these antibodies is a simplification of the normally richly enfolded subsynaptic membrane and a reduction of the amount of AChR. The AChR is a sodium channel that opens to generate a transient end-plate potential in response to a quantity of acetylcholine released by the nerve terminal. A reduction in the membrane concentration of AChR causes a reduction in both the rate of rise and the height of the end-plate potential. This leads to a slight time delay in the muscle membrane action potential, a phenomenon known as jitter, which is measurable in the electromyographic laboratory.[116] Occasionally the end-plate potential fails to reach threshold and that muscle fiber fails to contract. The statistical failure of a large number of such fibers in time is the basis for weakness and fatigue in a patient with myasthenia.

The treatment of myasthenia is gratifying to clinicians.[114-117] Anticholinesterase medications can control many of the symptoms and are sufficient treatment in many cases. Corticosteroids and occasionally azathioprine or other immunosuppressive medications are used when needed. Patients in crisis may need mechanical ventilation and tube feeding, and plasmapheresis provides the most rapid improvement. Thymectomy has been an important treatment since the 1940s. The thymus gland is frequently enlarged for age in patients with myasthenia. The occurrence of thymomas is also well recognized in this disease. The rationale for thymectomy and some of our experience at UCLA are described in detail.

The Myasthenic Syndrome

The myasthenic syndrome, or the Lambert-Eaton myasthenic syndrome, is another antibody-mediated disease of neuromuscular transmission.[118] In this disorder, antibodies are directed at voltage-dependent calcium channels on the presynaptic nerve terminals, which are necessary for the coupling of the depolarization of the nerve terminal to the release of acetylcholine. This reduction in acetylcholine release can be partially and transiently overcome by a rapid volley of nerve impulses—for example, after a brief isometric contraction. This potentiation is easily shown in the laboratory by measuring the compound action-potential response of muscle to a single nerve stimulation before and after 15 seconds of isometric contraction. The physiologic defect of the Lambert-Eaton myasthenic syndrome can be transferred passively to experimental animals with serum, and patients improve with either immune suppression or plasmapheresis. The syndrome is strongly associated with small-cell carcinoma of the lungs, especially in smokers. Small-cell carcinoma is neurodermally derived and contains the same type of calcium channels that are present in the motor nerve terminals.

Causes of Autoimmunity

The effects of various autoantibodies directed at each specific site of the motor unit cause the specific diseases just described. Why are these autoantibodies formed? If we knew the primary cause of the induction of these autoantibodies, we might devise better and more specific treatments.

What induces autoantibodies? There are three relevant areas of research: the genetics of the immune response, the immune networks, and the thymus in patients with myasthenia gravis.

Like a number of other autoimmune diseases, myasthenia gravis is associated with a higher-than-expected frequency of certain HLA haplotypes.[119,120] There is no simple genetic inheritance of myasthenia or other autoimmune diseases. Probably a number of genetic loci, possibly including B- and T-cell receptor genes, immunoglobulin variable region genes, and class I, II, and III MHC molecules, play a cumulative role in determining susceptibility to these conditions, as suggested by Newsom-Davis and co-workers for myasthenia gravis.[119] The associations of myasthenia with these and other markers suggest that there are genes that enhance the ability to produce the autoantibodies of myasthenia. So far there is little or no evidence for such associations in other diseases discussed in this section.

The immune network theory provides a framework for the production of anti-idiotypic (anti-Id) antibodies that may cross-react with autoantigens.[121] For example, anti-Id antibodies directed against some primary antimicrobial antibodies have anti-AChR activity.[122] This suggests a mechanism for the induction of the anticarbohydrate antibodies associated with motor neuron diseases and with demyelinating autoimmune neuropathies. Many viruses use cell-surface carbohydrates as receptors. Viruses may attach to the same carbohydrate structures recognized by the autoantibodies of these neurologic diseases. The proposed mechanism is that the virus recognizes the cell-surface receptor, the viral infection induces the antiviral antibody, the antiviral antibody recognizes the receptor binding site on the virus, the anti-Id antibody is produced against the antiviral antibody, and the anti-Id antibody cross-reacts with the cell-surface receptor and becomes an autoantibody.

There are many examples of this mechanism in which immunization with a virus, a hormone, or a drug induces antibodies directed at the cellular receptor for the virus, hormone, or drug.[123] We have evidence that the mechanism can operate in an experimental system of relevance to autoimmune neurologic disease.

Cytomegalovirus and Autoimmunity

Cytomegalovirus (CMV) is one of the most common viruses associated with the Guillain-Barré syndrome. The timing of paralysis suggests a postinfectious mechanism. Autoantibodies are found in human CMV infection and in experimental murine CMV infection of mice.[124] We studied the structure of the host-cell receptor for murine CMV. Attenuated murine CMV specifically recognizes the sequence of the sugars *N*-acetylglucosamine-galactose.[125] This is similar to the antigens of autoimmune demyelinating neuropathies. In experimental infection, mice first produce antiviral antibodies, with peak production at 21 days. At 36 days postinfection, antibodies directed at the receptor also appear. Analysis of a panel of antiviral and antireceptor monoclonal antibodies from mice indicated that some antibodies are produced that are antireceptor autoantibodies and are also anti-Id antibodies (react with the antiviral antibodies), as predicted by the mechanism just described. The identity of the human CMV receptor is not yet known, but it is possible that human CMV and other human viruses may induce autoantibodies by the type of anti-Id mechanism that we have shown in mice.

The Thymus in Myasthenia Gravis

In patients with myasthenia gravis, the thymus is frequently enlarged, and there is also an increased incidence of thymoma. The removal of the thymus is associated with an improved prognosis.[126] It has been estimated that the thymus is the source of a significant but small portion of anti-AChR production in patients. The presence of AChR-bearing myoepithelial cells in close proximity to immune lymphocytes in the thymus has been proposed as the milieu in which autoimmunity is induced.[119] Thymectomy is thus a strategy for potentially intervening early in the process of the induction of autoimmunity.

Role of Thymectomy in Myasthenia Gravis

DONALD G. MULDER, MD*: Nerve impulse transmission to muscle occurs at the acetylcholine receptor site by the release of acetylcholine, which is counterbalanced appropriately by postsynaptic cholinesterase. In patients with myasthenia gravis, many of these receptor sites are blocked, bound, or degraded by antibodies induced by events arising in the patients' own thymus glands. As a result of this autoimmune process, patients have a decreased number of normally functioning neuromuscular units and have symptoms of weakness and easy fatigability.

There are four ways that a myasthenic patient can be managed:

- Anticholinesterase medications, the most common being pyridostigmine bromide (Mestinon), to enhance the acetylcholine effect on the remaining functioning AChR;
- Immunosuppressive drugs, such as prednisone and azathioprine, to control the immune response;
- Plasmapheresis treatments to transiently decrease the amount of circulating antibody; and
- Thymectomy to remove the source of autoantibody induction.

Indications for Operation

In recent years we have recommended thymectomy earlier in the course of the disease for several reasons: surgical morbidity and mortality are low; many patients have progression of symptoms despite increasing dosages of anticholinesterase medications; intolerance to medications develops, especially as the dosages are increased; long-term side effects and complications of steroid therapy develop; plasmapheresis produces only a transient benefit; and reversibility of impaired function at the AChR sites becomes a concern once they have become blocked or bound by antibody. Even if spontaneous improvement should occur, it is known that such improvement or even remission is uncommon and short-lived.

Thus, the clinical diagnosis of myasthenia gravis—substantiated unequivocally by a positive test with edrophonium bromide (Tensilon), frequently (85%) with the finding of receptor site antibodies in the serum, and abnormal single fiber electromyography—warrants consideration of thymectomy even in those patients with ocular and mild generalized disease. The finding on routine chest roentgenograms of an anterior mediastinal mass, further delineated by a computed tomographic scan or magnetic resonance imaging, and the presence of antistriational muscle antibody (90%) provide strong evidence for the diagnosis of thymoma. The presence of a thymoma, which occurs in approximately 20% of patients with myasthenia, is another indication for operation.

*Professor, Department of Surgery, Division of Cardiothoracic Surgery, UCLA School of Medicine.

Perioperative Considerations

Most patients can be operated on when their symptoms are stable and under satisfactory medical control. In those with rapidly progressive disease that is difficult to control, the use of plasmapheresis has been of great help in stabilizing patients before an operation. Because only about 40% of the receptor site antibody is present in the serum at any one time, multiple treatments (weekly for four to six weeks) may be necessary to achieve the desired results. The transient but usually dramatic improvement in a patient's condition permits a much safer operation.

Total thymectomy is done through a median sternotomy incision, which gives optimum exposure to the bilobed structure lying directly behind the sternum. The lateral margins approach the phrenic nerves, which are carefully spared from injury as the entire gland and all mediastinal fat—which occasionally harbors aberrant remnants of thymic tissue—are removed. The cervical pedicles of the thymus, which are frequently contiguous with the inferior pole of the thyroid, are also removed.

Any thymoma should be excised with a generous margin of contiguous tissue and the entire thymus gland removed as well. Locally invasive thymomas should be managed aggressively by block resection of involved pericardium, lungs, and vascular structures because the results of surgical removal supplemented by irradiation, chemotherapy, or both, are surprisingly good.

Because many patients have limited ventilatory reserve, their anesthetic management and postoperative care are extremely important to minimize pulmonary complications. Most patients can be extubated in the immediate postoperative period, whereas some must be carefully weaned from the respirator after one or two days. A tracheostomy has rarely been necessary.

TABLE 3.—*Results of Thymectomy in 333 Patients With Myasthenia Gravis From 1954 to 1987*

	Patients					
	Men		Women		Total	
Result	No.	(%)	No.	(%)	No.	(%)
Remission	35	(38)	121	(50)	156	(47)
Improved	38	(41)	89	(37)	127	(38)
Operation benefited	73	(78)	210	(88)	283	(85)
Same	10		22		32	
Worse	1		1		2	
Dead*	9		7		16	
Total	93		240		333	

*2 operative deaths; mean follow-up 6.45 yr.

Results After Thymectomy

In recent years thymectomy has gained increasing acceptance as the most effective therapy for achieving sustained improvement in most patients with myasthenia gravis.[127-130] We reported our results at the UCLA Medical Center in 1983 with 249 patients[127] and again in 1989 with an additional 84 patients.[126] This 33-year experience with 333 patients is summarized in Table 3. Remission—no medication, no symptoms—was achieved in 121 of the 240 women (50%) and 35 of the 93 men (38%). Improvement—less medication, fewer symptoms—was noted in an additional 37% of the women and 41% of the men. Overall, 283 of the 333 patients (85%) benefited from the operation. Reoperation for recurrent invasive thymoma resulted in two operative deaths. Over a mean follow-up period of 6.45 years, there were 14 late deaths, which in most instances were caused by complications related to a progression or relapse of the patients' myasthenia.

An attempt was made in the recent five-year review of our experience[126] to identify factors that might predict a more favorable outcome after thymectomy. The presence of AChR antibodies in the patients' serum and a thymus gland containing areas of hyperplasia were found to be predictors of a favorable outcome.

Serum for determining AChR antibody levels was obtained from 76 of the 84 patients in the recent series. Antibodies were detected in 43 patients, of whom 19 (44%) achieved remission and 21 had improvement; hence, 40 patients (93%) benefited from the operation. In contrast, only 21 (64%) of the 33 patients in whom no antibody was detectable benefited from the operation. Nine (27%) achieved remission, and 12 showed improvement.

The presence of hyperplasia in the excised thymus gland was noted in 38 patients, of whom 20 (53%) achieved remission and 13 had improvement; hence, 33 (87%) benefited from the operation. A normal gland—one without specific diagnostic changes, such as atrophy or involution—was found in 35 patients and was associated with remission in 7 (20%) and improvement in 17, so that 24 patients (69%) benefited from the operation.

In the 11 patients with thymoma, 10 (90%) benefited from the operation in which the thymoma and all remaining thymus were excised. Three patients (27%) had remission and seven improved postoperatively.

The combination of hyperplasia of the thymus gland and the presence of AChR antibodies was a favorable prognosticator (Table 4). Of the 23 patients with this combination, 15 achieved remission (65%) and 7 had improvement; hence, 22 (96%) of the 23 benefited from the operation. In contrast, the 22 patients without AChR antibodies and without hyperplasia did not have as favorable a response; 14 (64%) benefited from the operation, and only 6 (27%) had remission.

TABLE 4.—*Thymus Disease and Acetylcholine Receptor Antibodies After Thymectomy in 76 Patients With Myasthenia Gravis From 1982 to 1987*

	Patients						
		Remission		Improved		Benefited From Operation	
Disease and Antibody (Ab) Status	Total No.	No.	(%)	No.	(%)	No.	(%)
Hyperplasia, Ab+	23	15	(65)	7	(30)	22	(96)
Hyperplasia, Ab−	11	3	(27)	4	(36)	7	(64)
No diagnostic changes, Ab+	10	1	(10)	8	(80)	9	(90)
No diagnostic changes, Ab−	22	6	(27)*	8	(36)	14	(64)*
Thymoma, Ab+	10	3	(30)	7	(70)	10	(100)

+ = positive, − = negative

*Difference between categories is $P<.05$.

Of the 11 patients with thymoma, all 10 with AChR antibodies benefited from the operation. Three patients (30%) achieved remission.

Based on our experience and that of others, it can be said that thymectomy is beneficial to most patients with myasthenia gravis. The operation has a low morbidity and mortality.

Although the outcome is not entirely predictable for any specific patients, predictors of success are a relatively young age with less severe disease of short duration in a patient who does not have an invasive thymoma but who has hyperplasia of the thymus and the presence of anti-AChR antibodies preoperatively.

REFERENCES

1. Prineas JW: The neuropathology of multiple sclerosis. *In* Vinken PJ, Bruyn GW, Klawans HL (Eds): Handbook of Clinical Neurology. New York, NY, Elsevier Science Publishing, 1985, pp 213-257

2. McFarlin DE, McFarland HF: Multiple sclerosis (1st of 2 pts). N Engl J Med 1982; 307:1183-1188

3. Gebarski SS, Gabrielsen TO, Gilman S, Knake JE, Latack JT, Aisen AM: The initial diagnosis of multiple sclerosis: Clinical impact of magnetic resonance imaging. Ann Neurol 1985; 17:469-474

4. Paty DW, McFarlin DE, McDonald WI: Magnetic resonance imaging and laboratory aids in the diagnosis of multiple sclerosis. Ann Neurol 1991; 29:3-5

5. Oger J, Kastrukoff L, O'Gorman M, Paty DW: Progressive multiple sclerosis: Abnormal immune functions in vitro and aberrant correlation with enumeration of lymphocyte subpopulations. J Neuroimmunol 1986; 12:37-48

6. Calder V, Owens S, Watson C, Feldmann M, Davison A: Multiple sclerosis: A localized immune disease of the central nervous system. Immunol Today 1989; 10:99-103

7. Hafler DA, Weiner HL: Multiple sclerosis: A CNS and systemic autoimmune disease. Immunol Today 1989; 10:104-107

8. Unanue ER: The major histocompatibility complex and immunogenetics: Antigen presenting function of the macrophage. *In* Paul WE, Fathman CG, Metzger H (Eds): Annual Review of Immunology, Vol 2. Palo Alto, Calif, Annual Reviews, 1984, pp 395-423

9. Davis MM, Bjorkman PJ: T-cell antigen receptor genes and T-cell recognition. Nature 1988; 334:395-398

10. Brown JH, Jardetzky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC: A hypothetical model of foreign antigen binding site of class II histocompatibility molecules. Nature 1988; 334:845-847

11. Zamvil SS, Mitchell DJ, Moore AC, Kitamura K, Steinman L, Rothbard JB: T-cell epitope of the autoantigen myelin basic protein that induced encephalomyelitis. Nature 1986; 324:258-260

12. Ando D, Clayton J, Kono D, Urban J, Sercarz EE: Encephalitogenic T-cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are the Th-1 lymphokine subtype. Cell Immunol 1989; 168:132-143

13. Kono DH, Urban JL, Horvath SJ, Ando DG, Saavedra RA, Hood L: Two minor determinants of myelin basic protein induce experimental allergic encephalomyelitis in SJL/J mice. J Exp Med 1988; 168:213-227

14. Clayton JP, Gammon GM, Ando DG, Kono DH, Hood L, Sercarz EE: Peptide-specific prevention of experimental allergic encephalomyelitis, neonatal tolerance induced to the dominant T-cell determinant of myelin basic protein. J Exp Med 1989; 169:1681-1693

15. Sayetta RB: Theories of the etiology of multiple sclerosis: A critical review. J Clin Lab Immunol 1986; 21:55-70

16. Dhib-Jalbut S, McFarlin DE: Macrophages, microglia and other antigen presenting cells in neurological disorders. Prog Neuroendocrin Immunol 1989; 2:86-94

17. Hofman FM, von Hanwehr RI, Dinarello CA, Mizel SB, Hinton D, Merrill JE: Immunoregulatory molecules and IL 2 receptors identified in multiple sclerosis brain. J Immunol 1986; 136:3239-3245

18. Traugott U, Lebon P: Interferon γ and Ia antigen are present on astrocytes in active chronic multiple sclerosis lesions. J Neurol Sci 1988; 84:257-264

19. Woodroofe MN, Bellamy AS, Feldmann M, Davison AN, Cuzner ML: Immunocytochemical characterization of the immune reaction in the central nervous system in multiple sclerosis—Possible role for microglia in lesion growth. J Neurol Sci 1986; 74:135-152

20. Oldstone MBA: Molecular mimicry and autoimmune disease. Cell 1987; 50:819-820

21. Wekerle H, Linington C, Lassmann H, Meyermann R: Cellular immune reactivity within the CNS. Trends Neurosci 1986; 9:271-277

22. Hafler DA, Benjamin DS, Burks J, Weiner HL: Myelin basic protein and proteolipid protein reactivity of brain and cerebrospinal fluid-derived T-cell clones in multiple sclerosis and postinfectious encephalomyelitis. J Immunol 1987; 139:68-72

23. Hafler DA, Duby AD, Lee SJ, Benjamin D, Seidman JG, Weiner HL: Oligoclonal T lymphocytes in the cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1988; 167:1313-1322

24. Merrill JE, Kagan JM, Schmid I, Strom SR, Quan SG, Chen ISY: T-cell lines established from multiple sclerosis cerebrospinal fluid T-cells using human retroviruses. J Neuroimmunol 1989; 21:213-226

25. Rotteveel FTM, Kuenen B, Kokkelink I, Meager A, Lucas CJ: Relative increase of inflammatory CD4+ T-cells in the cerebrospinal fluid of multiple sclerosis patients and control individuals. Clin Exp Immunol 1990; 79:15-20

26. Greenstein JI, McFarland HF, Richert JR: Characterization of antigen-specific T-cells in multiple sclerosis twins with elevated proliferative responses to measles virus. J Immunol 1986; 137:546-550

27. Estes ML, Rudick RA, Barnett GH, Ransohoff RM: Stereotactic biopsy of an active multiple sclerosis lesion—Immunocytochemical analysis and neuropathologic correlation with magnetic resonance imaging. Arch Neurol 1990; 47:1299-1303

28. Allegretta M, Nicklas JA, Sriram S, Albertini RJ: T cells responsive to myelin basic protein in patients with multiple sclerosis. Science 1990; 247:718-721

29. Oksenberg JR, Stuart S, Begovich AB, et al: Limited heterogeneity of rearranged T-cell receptor Vα transcripts in brains of multiple sclerosis patients. Nature 1990; 345:344-346

30. Seboun E, Robinson MA, Doolittle TH, Ciulla TA, Kindt TJ, Hauser SL: A susceptibility locus for multiple sclerosis is limited to the T-cell receptor β chain complex. Cell 1989; 57:1095-1100

31. Merrill JE, Mohlstrom C, Uittenbogaart C, Kermaniarab V, Ellison GW, Myers LW: Response to and production of interleukin 2 by peripheral blood and cerebrospinal fluid lymphocytes of patients with multiple sclerosis. J Immunol 1984; 133:1931-1937

32. Selmaj K, Nowak Z, Tchorzewski H: Interleukin 1 and interleukin 2 production by peripheral blood mononuclear cells in multiple sclerosis patients. J Neurol Sci 1988; 85:67-76

33. Brajczewska-Fischer W, Iwińska B, Kruszewska J, Korlak J, Członkowska A: Interleukin 1 and 2 production by peripheral blood mononuclear cells in subacute sclerosing panencephalitis and exacerbation of multiple sclerosis. Acta Neurol Scand 1989; 80:390-393

34. Trotter JL, Clifford DB, McInnis JE, et al: Correlation of immunological studies and disease progression in chronic progressive multiple sclerosis. Ann Neurol 1989; 25:172-178

35. Gallo P, Piccinno M, Pagni S, Tavolato B: Interleukin 2 levels in serum and cerebrospinal fluid of multiple sclerosis patients. Ann Neurol 1988; 24:795-797

36. Gallo P, Piccinno MG, Pagni S, et al: Immune activation in multiple sclerosis: Study of IL2, SIL2R, and γ-IFN levels in serum and cerebrospinal fluid. J Neurol Sci 1989; 92:9-15

37. Trotter JL, Vanderveen RC, Clifford DB: Serial studies of serum interleukin 2 in chronic progressive multiple sclerosis patients: Occurrence of bursts and effect of cyclosporine. J Neuroimmunol 1990; 28:9-14

38. Kittur SD, Kittur DS, Soncrant TT, et al: Soluble interleukin 2 receptors in cerebrospinal fluid from individuals with various neurological disorders. Ann Neurol 1990; 28:168-173

39. Wong GHW, Clark-Lewis I, Harris AW, Schrader JW: Effect of cloned interferon gamma on expression of H2 and Ia antigens on cell lines of hemopoietic, lymphoid, epithelial, fibroblastic, and neuronal origin. Eur J Immunol 1984; 14:52-56

40. Chung IY, Benveniste EN: Tumor necrosis factor-α production by astrocytes—Induction by lipopolysaccharide, IFNγ, and IL-1β. J Immunol 1990; 144:2999-3007

41. Fierz W, Endler B, Reske K, Wekerle H, Fontana A: Astrocytes as antigen-presenting cells—I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. J Immunol 1985; 134:3785-3793

42. Benveniste EN, Sparacio SM, Bethea JR: Tumor necrosis factor-α enhances interferon-γ-mediated class II antigen expression on astrocytes. J Neuroimmunol 1989; 25:209-219

43. Massa PT, Schimpl A, Wecker E, ter Meulen V: Tumor necrosis factor amplifies measles virus-mediated Ia induction on astrocytes. Proc Natl Acad Sci USA 1987; 84:7242-7245

44. Traugott U: Multiple sclerosis: Relevance of class I and class II MHC-expressing cells to lesion development. J Neuroimmunol 1987; 16:283-302

45. Hirsch RL, Panitch HS, Johnson KP: Lymphocytes from multiple sclerosis patients produce elevated levels of gamma interferon in vitro. J Clin Immunol 1985; 5:386-389

46. Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wietzerbin J: Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: Do cytokines trigger off exacerbations? Acta Neurol Scand 1988; 78:318-323

47. Olsson T, Zhi WW, Höjeberg B, et al: Autoreactive T lymphocytes in multiple sclerosis is determined by antigen-induced secretion of interferon γ. J Clin Invest 1990; 86:981-985

48. Panitch HS, Hirsch RL, Haley AS, Johnson KP: Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet 1987; 1:893-895

49. Panitch HS, Hirsch RL, Schindler J, Johnson KP: Treatment of multiple sclerosis with gamma interferon: Exacerbations associated with activation of the immune system. Neurology 1987; 37:1097-1102

50. Prineas JW, Graham JS: Multiple sclerosis: Capping of surface immunoglobulin G on macrophages engaged in myelin breakdown. Ann Neurol 1981; 10:149-158

51. Dore-Duffy P, Donaldson JO, Koft T, Longo M, Perry W: Prostaglandin release in multiple sclerosis: Correlation with disease activity. Neurology 1986; 36:1587-1590

52. Merrill JE, Gerner RH, Myers LW, Ellison GW: Regulation of natural killer cell cytotoxicity by prostaglandin E in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis and other neurological diseases—I. Association between the amount of prostaglandin produced, natural killer, and endogenous interferon. J Neuroimmunol 1983; 4:223-238

53. Freeman BA, Crapo JD: Biology of disease: Free radicals and tissue injury. Lab Invest 1982; 47:412-426

54. Hammann KP, Nix W, Dierich MP, Hopf HC: Multiple sclerosis patients show an increased spontaneous activity of their peripheral blood monocytes as measured by chemiluminescence. Acta Neurol Scand 1983; 68:151-156

55. Hunter MIS, Nlemadim BC, Davidson DLW: Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. Neurochem Res 1985; 10:1645-1652

56. Feldberg W, Gupta KP: Pyrogen, fever, and prostaglandin-like activity in cerebrospinal fluid. J Physiol 1973; 228:41-53

57. Bernheim HA: Is prostaglandin E_2 involved in the pathogenesis of fever? Effects of interleukin 1 on the release of prostaglandins. Yale J Biol Med 1986; 59:141-148

58. Dinarello CA, Cannon JG, Wolff SM, et al: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J Exp Med 1986; 163:1433-1450

59. Philip R, Epstein LB: Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, interferon, and interleukin 1. Nature 1986; 323:86-89

60. Waxman SG: Clinicopathological correlations in multiple sclerosis and related

diseases, *In* Waxman SG, Ritchie JM (Eds): Demyelinating Diseases: Basic and Clinical Electrophysiology. New York, NY, Raven Press, 1981, pp 169-182

61. Merrill JE, Strom SR, Ellison GW, Myers LW: In vitro study of mediators of inflammation in multiple sclerosis. J Clin Immunol 1989; 9:84-96

62. Selmaj K, Nowak Z, Tchórzewski H: Multiple sclerosis: Effect of myelin basic protein on interleukin 1, interleukin 2 production and interleukin 2 receptor expression in vitro. Clin Exp Immunol 1988; 72:428-433

63. Franciotta DM, Grimaldi LME, Martino GV, et al: Tumor necrosis factor in serum and cerebrospinal fluid of patients with multiple sclerosis. Ann Neurol 1989; 26:787-789

64. Hofman FM, Hinton DR, Johnson K, Merrill JE: Tumor necrosis factor identified in multiple sclerosis brain. J Exp Med 1989; 170:607-612

65. Leist TP, Frei K, Kam-Hansen S, Zinkernagel RM, Fontana A: Tumor necrosis factor-α in cerebrospinal fluid during bacterial, but not viral meningitis—Evaluation in murine model of infections and in patients. J Exp Med 1988; 167:1743-1748

66. Nodal D, Leppert D, Frei K, Gallo P, Lamche H, Fontana A: Tumor necrosis factor-α in infectious meningitis. Arch Dis Child 1989; 64:1274-1279

67. Gallo P, Piccinno MG, Krzalic L, Tavolato B: Tumor necrosis factor alpha (TNFα) and neurological diseases—Failure in detecting TNFα in the cerebrospinal fluid from patients with multiple sclerosis, AIDS dementia complex, and brain tumours. J Neuroimmunol 1989; 23:41-44

68. Hauser SL, Doolittle TH, Lincoln R, Brown RH, Dinarello CA: Cytokine accumulations in CSF of multiple sclerosis patients: Frequent detection of interleukin 1 and tumor necrosis factor but not interleukin 6. Neurology 1990; 40:1735-1739

69. Lieberman AP, Pitha PM, Shin HS, Shin ML: Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proc Natl Acad Sci USA 1989; 86:6346-6352

70. Sawada M, Kondo N, Suzumura A, Marunouchi T: Production of tumor necrosis factor-α by microglia and astrocytes in culture. Brain Res 1989; 491:394-397

71. Fontana A, Kristensen F, Dubs R, Gemsa D, Weber E: Production of prostaglandin E and interleukin 1-like factor by cultured astrocytes and C6 glioma cells. J Immunol 1982; 129:2413-2419

72. Giulian D, Lachman LB: Interleukin 1 stimulation of astroglial proliferation after brain injury. Science 1985; 228:497-499

73. Lachman LB, Brown DC, Dinarello CA: Growth-promoting effect of recombinant interleukin 1 and tumor necrosis factor for a human astrocytoma cell line. J Immunol 1987; 138; 2913-2916

74. Selmaj KW, Farooq M, Norton WT, Raine CS, Brosnan CF: Proliferation of astrocytes in vitro in response to cytokines: A primary role for tumor necrosis factor. J Immunol 1990; 144:129-135

75. Merrill JE: The effect of IL-1 and TNFα on astrocytes, microglia, oligodendrocytes, and glial precursors in vitro. Dev Neurosci 1991; 13:130-137

76. Merrill JE, Zimmerman RP: Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGF. GLIA 1991; 4:327-331

77. Babinski J: Recherches sur l'anatomie pathologique de la sclérose en plaque et étude comparative des diverses varié de scléroses de la moelle. Arch Physiol 1985; 5-6:186-207

78. Robbins DS, Shirazi Y, Drysdale BE, Lieberman A, Shin HS, Shin ML: Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. J Immunol 1987; 139:2593-2597

79. Selmaj KW, Raine CS: Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988; 23:339-346

80. Tsunawaki S, Sporn M, Ding A, Nathan C: Deactivation of macrophages by transforming growth factor. Nature 1988; 334:260-262

81. Rack MK, Dhib-Jalbut S, Cannella B, Albert PS, Raine CS, McFarlin DE: Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor beta. J Immunol 1991; 146:3012-3017

82. Ellison GW, Myers LW: Immunosuppressive drugs in multiple sclerosis: Pro and con. Neurology 1980; 80:28-32

83. Myers LW, Fahey JL, Moody DJ, Mickey MR, Frane MV, Ellison GW: Cyclophosphamide 'pulses' in chronic progressive multiple sclerosis—A preliminary clinical trial. Arch Neurol 1987; 44:828-832

84. Mickey MR, Ellison GW, Fahey JL, Moody DJ, Myers LW: Correlation of clinical and immunologic states in multiple sclerosis. Arch Neurol 1987; 44:371-375

85. Multiple Sclerosis Study Group: The efficacy and toxicity of cyclosporine in chronic progressive multiple sclerosis: A randomized, double blinded, placebo-controlled clinical trial. Ann Neurol 1990; 27:591-605

86. Vandenbark AA, Hashim G, Offner H: Immunization with a synthetic T-cell receptor γ-region peptide protects against experimental autoimmune encephalomyelitis. Nature 1989; 341:541-544

87. Weiner HL, Cohen IR, Hafler DA: Immunologic effects following T-cell vaccination in chronic progressive multiple sclerosis (Abstr). Neurology 1989; 39:172

88. Adorini L, Barnaba V, Bona C, et al: New perspectives on immunointervention in autoimmune diseases. Immunol Today 1990; 11:383-386

89. Hafler DA, Weiner HL: Immunosuppression with monoclonal antibodies in multiple sclerosis. Neurology 1988; 38:42-47

90. Knobler RL, Panitch HS, Braheny SL, et al: Systemic α-interferon therapy of multiple sclerosis. Neurology 1984; 34:1273-1279

91. Jacobs L, Slazar AM, Herndon R, et al: Multicenter double blind study of effect of intrathecally administered natural human fibroblast interferon on exacerbations of multiple sclerosis. Lancet 1986; 2:11-13

92. Milanese C, Salmaggi A, La Mantia L, Corridori F, Nespolo A: Intrathecal β-interferon in multiple sclerosis. Lancet 1988; 2:563-564

93. Barna BP, Chou SM, Yen-Lieberman B, Ransohoff RM: Interferon beta impairs induction of HLA DR antigen expression on cultured adult human astrocytes. J Neuroimmunol 1989; 23:45-53

94. Noronha A, Toscas A, Jensen M: Beta-interferon augments suppressor-cell function in progressive multiple sclerosis. Neurology 1988; 38(suppl 1):256-263

95. Panitch HS, Folus JS, Johnson KP: Recombinant β interferon inhibits γ interferon production in multiple sclerosis (Abstr). Ann Neurol 1987; 22:139

96. Lorish TR, Thorsteinsson G, Howard FM: Stiff-man syndrome updated. Mayo Clin Proc 1989; 64:629-636

97. Roobol TH, Kazzaz BA, Vecht CJ: Segmental rigidity and spinal myoclonus as a paraneoplastic syndrome. J Neurol Neurosurg Psychiatry 1987; 50:628-631

98. Diniz MA, Cunha L, Goncalves F, Oliveira C: Two cases of stiff man syndrome with tremor. Rev Neurol (Paris) 1986; 142:918-920

99. Solimena M, Folli F, Denis-Donini S, et al: Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes. N Engl J Med 1988; 318:1012-1020

100. Gorin F, Baldwin B, Tait R, Pathak R, Seyal M, Mugnaini E: Stiff-man syndrome: A GABAergic autoimmune disorder with autoantigenic heterogeneity. Ann Neurol 1990; 28:711-714

101. Solimena M, Folli F, Aparisi R: Autoantibodies to GABAergic neurons and pancreatic beta cells in stiff-man syndrome. N Engl J Med 1990; 322:1555-1560

102. Brashear HR, Phillips LH: Autoantibodies to GABAergic neurons and response to plasmapheresis in stiff man syndrome. Neurology 1991; 41:1588-1592

103. Williams DB, Wildebank AJ: Motor neuron disease (amyotrophic lateral sclerosis). Mayo Clin Proc 1991; 66:4-82

104. Drachman DB, Kuncl RW: Amyotrophic lateral sclerosis: An unconventional autoimmune disease? Ann Neurol 1989; 26:269-274

105. Pestronk A, Cornblath D, Ilyas AA, et al: A treatable multifocal motor neuropathy with antibodies to GM1 ganglioside. Ann Neurol 1988; 24:73-78

106. Pestronk A, Chaudhry V, Feldman EL, et al: Lower motor neuron syndromes defined by patterns of weakness, nerve conduction abnormalities, and high titers of antiglycolipid antibodies. Ann Neurol 1990; 27:316-326

107. Pestronk A, Adams RN, Kuncl RW, Drachman DB, Clawson LL, Cornblath DR: Differential effect of prednisone and cyclophosphamide on autoantibodies in human neuromuscular disorders. Neurology 1989; 39:628-633

108. Nobile-Orazio E, Baldini L, Barbieri S, et al: Treatment of patients with neuropathy and anti-MAG IgM M-proteins. Ann Neurol 1988; 24:93-97

109. Koski CL: Guillain Barré syndrome. Autoimmun Forum Neurol 1989; 1:2-4

110. Barohn RJ, Kissel JT, Warmotts JR, Mendell JR: Chronic inflammatory demyelinating polyradiculoneuropathy: Clinical characteristics, course, and recommendations for diagnostic criteria. Arch Neurol 1989; 46:878-884

111. Quarles RH: Antibodies to complex carbohydrates that may mediate neuropathy. Prog Neuroendocrinimmunol 1989; 2:109-119

112. Latov N: Neuropathic syndromes associated with autoreactive IgM antibodies. Autoimmun Forum Neurol 1989; 1:2-4

113. Trojaborg W, Galassi G, Hays AP, Lovelace RE, Alkaitis M, Latov N: Electrophysiologic study of experimental demyelination induced by serum of patients with IgM M proteins and neuropathy. Neurology 1989; 39:1581-1586

114. Levin KH, Richman DP: Myasthenia gravis. Clin Aspects Autoimmun 1989; 4:23-31

115. Drachman DB, Sesilva S, Ramsay D, Pestronk A: Humoral pathogenesis of myasthenia gravis. Ann NY Acad Sci 1987; 505:90-105

116. Sanders D: The electrodiagnosis of myasthenia gravis. Ann NY Acad Sci 1987; 505:539-556

117. Rowland LP: Therapy in myasthenia gravis: Introduction. Ann NY Acad Sci 1987; 505:566-567

118. Vincent A, Lang B, Newsom-Davis J: Autoimmunity to the voltage-gated calcium channel underlies the Lambert-Eaton myasthenic syndrome, a paraneoplastic disorder. Trends Neurol Sci 1989; 12:486-502

119. Newsom-Davis J, Willcox N, Schluep M, et al: Immunological heterogeneity and cellular mechanisms in myasthenia gravis. Ann NY Acad Sci 1987; 505:12-16

120. Keesey J, Naiem F, Lindstrom J, Roe D, Herrmann C, Walford R: Acetylcholine receptor antibody and HLA-B8 antigen in myasthenia gravis. Arch Neurol 1982; 39:73-77

121. Williams WV, Guy HR, Cohen JA, Weiner DB, Greene MI: Molecular and immunologic analyses of a functional internal image formed by an antireceptor antibody. Ann Inst Pasteur/Immunol 1988; 139:9-75

122. Dwyer DS, Vakil M, Bradley RJ, Oh SJ, Kearney JF: A possible cause of myasthenia gravis: Idiotypic networks involving bacterial antigens. Ann NY Acad Sci 1987; 505:461-471

123. Krah DL, Choppin PW: Mice immunized with measles virus develop antibodies to a cell surface receptor for binding virus. J Virol 1988; 62:1565-1572

124. Bartholomaeus WN, O'Dinohue H, Foti D, et al: Multiple autoantibodies following cytomegalovirus infection: Virus distribution and specificity of autoantibodies. Immunology 1988; 64:397-405

125. Ravindranath R, Graves MC: Attenuated murine cytomegalovirus binds to *N*-acetylglucosamine and shift to virulence may involve recognition of sialic acids. J Virol 1990; 64:5430-5440

126. Mulder DG, Graves MC, Herrmann C: Thymectomy for myasthenia gravis: Recent observations and comparisons with past experience. Ann Thorac Surg 1989; 48:551-555 [see comments 1990; 49:688]

127. Mulder DG, Herrmann C Jr, Keesey J, Edwards H: Thymectomy for myasthenia gravis. Am J Surg 1983; 146:61-66

128. Genkins G, Kornfeld P, Papatestas AE, Bender AN, Matta RJ: Clinical experience in more than 2000 patients with myasthenia gravis. Ann NY Acad Sci 1987; 505:500-514

129. Papatestas AE, Genkins G, Kornfeld P, et al: Effects of thymectomy in myasthenia gravis. Ann Surg 1987; 206:79-88

130. Jaretzki A, Penn AS, Younger DS, et al: Maximal thymectomy for myasthenia gravis. J Thorac Cardiovasc Surg 1988; 95:747-757

EXHIBIT H

Topics in Primary Care Medicine

Current Pharmacologic Treatment of Multiple Sclerosis Symptoms

PETER-BRIAN ANDERSSON, MBChB, BSC(MED)(HONS),DPhil, *and*
DONALD E. GOODKIN, MD, *San Francisco, California*

About 350,000 persons in the United States have multiple sclerosis, and primary care physicians are often called on to provide symptomatic therapy for these patients. We review our current pharmacologic approach to the management of multiple sclerosis exacerbations and the symptoms of spasticity, fatigue, bladder and bowel involvement, neurobehavioral complaints, pain syndromes, dystonic spasms, and tremor and ataxia.

(Andersson PB, Goodkin DE: Current pharmacologic treatment of multiple sclerosis symptoms. West J Med 1996; 165:313–317)

Multiple sclerosis is the most common cause of neurologic disability affecting young adults in the Northern Hemisphere. It is estimated that 350,000 persons in the United States are affected with this disease, and primary care physicians are often called on to provide these patients with symptomatic therapy. We review our current pharmacologic approach to the management of multiple sclerosis exacerbations and symptoms that frequently occur in otherwise clinically stable patients.* Multidisciplinary care and disease-modifying therapies, equally important topics, are reviewed elsewhere.[1,2]

Acute Exacerbations

Exacerbations of multiple sclerosis consist of the appearance of new, or worsening of old, clinical signs or symptoms that last more than 24 hours in the absence of fever or infection. Clinical deterioration in conjunction with fever or infection is considered to be a "pseudoexacerbation." Pseudoexacerbations should be considered in any patient with multiple sclerosis having acute or subacute clinical deterioration, and the source of fever or infection should be identified and treated before considering the use of glucocorticosteroids.

The use of both glucocorticosteroids[3,4] and corticotropin[5,6] reduces the intensity and duration of the neurologic disability during relapses, though the intravenous (IV) administration of 1 gram of methylprednisolone sodium succinate each day for three to five days, followed by oral prednisone therapy begun at 60 mg and tapered gradually during the subsequent 11 to 18 days, is more efficacious than the intramuscular administration of corticotropin.[5–7] Preliminary evidence suggests similar efficacy when 1 gram of methylprednisolone is administered orally, and this route of administration appears to be well tolerated.[8] There is currently no consensus as to the dose, route of administration, or duration of steroid therapy for multiple sclerosis relapses, though data for clinical benefit[3–6] exist with high doses only, and reductions in gadolinium contrast enhancement of acute plaques are dose dependent.[9] Further, there is no evidence that steroid treatment of multiple sclerosis relapses nor long-term (maintenance) steroid therapy has any effect on the ultimate course of the disease (reviewed by Myers).[10] It is our current practice to treat only acute exacerbations that result in substantial disability with 1 gram of methylprednisolone given IV for 3 days, followed by 60 mg of prednisone administered orally and the dose tapered over 11 days. Until a larger clinical experience is published, we remain reluctant to treat multiple sclerosis patients with 1 gram of oral methylprednisolone because of lingering concerns regarding toxicity.

The therapeutic benefits of glucocorticosteroids in acute exacerbations are poorly understood, but are currently postulated to be attributable to one or more of the following mechanisms of action:

- Reduced blood-brain barrier permeability and resulting tissue edema,[9,11]
- Decreased intrathecal immunoglobulin G synthesis and complement activation,[10]
- Reduced proinflammatory cytokine production,[12]
- Immunosuppression by transforming growth factor β,[13] and
- Reduced expression of adhesion molecules on vascular endothelium.[14]

Patients with a first episode of isolated optic neuritis appear to be at a reduced risk of multiple sclerosis

*See also the editorial by L. B. Krupp, MD, "Advances in the Treatment of Multiple Sclerosis," on pages 320–321 of this issue.

From the University of California, San Francisco, School of Medicine (UCSF)–Mount Zion Multiple Sclerosis Center.
Reprint requests to Donald E. Goodkin, MD, Medical Director, UCSF–Mount Zion Multiple Sclerosis Center, 1600 Divisadero St, San Francisco, CA 94115.

developing for the following two years when treated with IV methylprednisolone, 250 mg every six hours for three days, followed by a tapering course of oral prednisone. This benefit is not observed in similar patients treated with 1 mg per kg of body weight of oral prednisone for 11 days.[15] The mechanism for this apparent treatment effect is unknown, and it has been postulated that glucocorticosteroids reduce T-cell reactivity to immunogenic myelin peptides that emerge after an initial episode of demyelination.[16] It is our practice to treat monosymptomatic optic neuritis with a regimen of 1 gram of IV methylprednisolone each day for three days, followed by prednisone, 60 mg administered orally each day for six days, and the dose tapered by 10 mg per day thereafter.

Spasticity

Spasticity is characterized by a velocity-dependent increase in resistance to passive or active movement of muscles. Spasticity is generally associated with weakness, hyperreflexia, extensor plantar responses, spontaneous muscle spasms, and limb stiffness. Physical therapy—daily active and passive range-of-motion exercises—and splinting are essentials of management to limit the development of contractures.[17]

Before initiating pharmacologic therapy for spasticity, it is important to recognize that limb stiffness often provides support for ambulation. Patients with spasticity that is associated with hip flexor weakness may lose their ability to ambulate if spasticity is treated too aggressively. It is also important to consider the possibilities of subclinical urinary tract infection and bladder or bowel distention before initiating therapy because these conditions may aggravate preexisting spasticity.

We restrict the treatment of spasticity to ambulatory patients without severe hip flexor weakness, those in whom treatment will facilitate personal care (for example, turning in bed or intermittent urethral catheterization), and patients who have painful spasms. We begin therapy with baclofen, 5 mg two or three times each day, and increase this dose by 5 mg every three days as tolerated. Frequent telephone contact with the patient is helpful in titrating the dosage. Doses approximating 30 mg each day are generally well tolerated, whereas higher doses increase the likelihood of adverse symptoms such as weakness, drowsiness, and leg swelling. Many patients require and tolerate daily doses approximating 80 mg, and occasionally a patient may respond only at higher doses. Patients treated with baclofen should be monitored for possible asymptomatic elevations of aminotransferase levels, and patients with compromised renal function may require adjusted dosage. The abrupt termination of therapy has been associated with agitated behavior, paranoid ideation, and seizures. Optimal results are achieved when pharmacotherapy is coupled with muscle stretching exercises supervised by a physical or occupational therapist.[17] Supplemental oral doses of diazepam, 1 to 2 mg, or clonidine, 0.1 to 0.2 mg, administered two or three times each day are also well tolerated and may be used to advantage to potentiate the therapeutic effects of baclofen.[18] Although the use of dantrolene sodium is often effective in patients with spasticity resulting from central nervous system trauma, its benefits in patients with multiple sclerosis are almost always overshadowed by unwanted weakness and sedation.

Intrathecal baclofen administered by a subcutaneous self-contained pump represents a reasonable alternative route for patients with severe spasticity that is unresponsive to oral therapies discussed earlier. This route of administration achieves fourfold higher cerebrospinal fluid drug concentrations while using only 1% of the oral dosage generally required for a treatment effect. A review of this experience suggests that more than 95% of patients treated with intrathecal baclofen have abatement or abolition of spasticity and spasms.[19] Surgical implantation of the self-contained pump, occasional mechanical or connecting tube malfunction, and cost represent obstacles to the more widespread use of this therapeutic application.

Bladder Symptoms

About 75% of patients with multiple sclerosis have symptoms of bladder dysfunction.[20] Symptoms of urgency, frequency, and urge incontinence are generally due to detrusor spasticity, whereas hesitancy, interruption of the urinary stream, or urine retention are more frequently associated with detrusor hypotonicity and incomplete relaxation of the internal and external sphincters during detrusor contraction (sphincter dyssynergia). Distinguishing these two types of bladder dysfunction is essential when attempting to provide effective therapy.

Measuring the amount of urine voided and the postvoiding volume of urine remaining usually enables the distinction to be made between bladder spasticity and hypotonicity. It is our practice to catheterize all symptomatic patients to determine the postvoiding residual volume and to exclude the possibility of a urinary tract infection as the cause of symptoms. Patients who void volumes of less than 200 to 300 ml and who have residual volumes of less than 100 ml generally have detrusor spasticity and often will have a reduction in symptoms with anticholinergic agents. We favor the use of oxybutynin chloride in doses of 2.5 to 5.0 mg orally two to three times each day, although the administration of propantheline bromide, 15 mg orally three to four times each day, is equally effective. Both medications are well tolerated, although dryness of the mouth and constipation are commonly experienced. Patients who void volumes of greater than 500 ml and who have postvoiding volumes of more than 100 ml generally have a hypotonic detrusor muscle, and those with residual volumes in excess of 200 ml often have associated sphincter dyssynergia. These patients are often responsive to intermittent self-catheterization,[21,22] a procedure easily taught and, in our experience, more effective than administering terazosin hydrochloride, 5 mg orally three to four times each day. Clinicians who lack the

resources to teach intermittent self-catheterization techniques should consider referring symptomatic patients to a multiple sclerosis center or a urologist. We refer to a urologist all patients with recurrent urinary tract infections or incontinence that does not respond to these measures.

Bowel Symptoms

Constipation, evacuation urgency, and incontinence occur in about 50% of patients with multiple sclerosis.[23] These symptoms are associated with abnormal colonic pressures, absent postprandial colonic motility, and functional outlet obstruction during defecation by an impaired puborectal muscle and anal sphincter relaxation.[24] Dietary adjustments with regular high-fiber meals, bulk formers, laxatives, enemas, timed voidings, and digitalization of the anus often alleviate symptoms. Rectal bags are occasionally useful, and diversionary procedures are rarely if ever indicated.

Fatigue

Fatigue occurs in about 75% of patients with multiple sclerosis and is typically worse during afternoon hours and with increased ambient temperature.[25] It is distinct from the fatigue seen in depression and sleep disorders,[26] and, in contrast to fatigue in normal controls, that in patients with multiple sclerosis is overwhelming and often requires the patient to sit, lie down, or sleep. The fatigue of this disorder correlates poorly with the degree of functional impairment shown on neurologic examination.[27,28]

Drug intervention coupled with the training of energy conservation techniques is often helpful in managing this symptom.[29] Amantadine hydrochloride, given orally in dosages of 100 mg once or twice each day, is effective in 41% to 62% of patients.[26-28] Other effective medications include pemoline, 37.5 mg, or fluoxetine hydrochloride, 10 to 20 mg, administered orally once or twice each day. Anxiety and insomnia are occasional adverse side effects. There is anecdotal evidence that patients who respond to these agents do so within several days and are unlikely to respond to higher doses. A sustained response in some patients may be facilitated by treatment regimens that include one-week drug holidays every three to four weeks.

Depression and Inappropriate Expressions of Emotion

Bipolar affective disease, anxiety disorder, and depression are more common in patients with multiple sclerosis than in age- and sex-matched groups.[30-34] Whether these conditions are directly attributable to cerebral plaques or are consequences of the psychological stresses associated with multiple sclerosis are not known. Therapy for depression in patients with multiple sclerosis is similar to that for the general population. We administer amitriptyline starting at a dosage of 25 mg each evening before bed and increasing this dose by 25 mg each week to a maintenance dose of 75 to 100 mg each day. The anticholinergic properties of this drug are also helpful to patients with symptoms of bladder spasticity or chronic pain. Patients with severe fatigue tend to tolerate this drug poorly and may respond more favorably to fluoxetine, 10 to 20 mg administered orally each morning. Patients with multiple sclerosis are at increased risk for suicide compared with the age-matched general population.[35] They should be questioned directly about symptoms suggestive of depression at each medical evaluation, and those who are unresponsive to initial treatment efforts should be referred for psychiatric consultation.

Inappropriate expressions of emotion (pathologic laughter or weeping) occur in 10% of patients[33] and are generally responsive to the oral administration of amitriptyline at a dosage of 10 to 75 mg each day.[31] Anecdotal experience suggests that oral fluoxetine in dosages of 10 to 20 mg each day is equally effective.

Pain Syndromes

Pain is reported to occur in about 55% of patients with multiple sclerosis.[36] Female patients and patients older than 40 years appear to be at an increased risk. About 80% of these patients have chronic pain syndromes. These syndromes are characterized by extremity dysesthesia, back pain, leg spasms, and abdominal pain and often occur in conjunction with myelopathy.

Chronic Pain

Chronic dysesthetic extremity pain, the most common of the chronic pain syndromes, is generally characterized as a burning or raw sensation that worsens at night. Administering tricyclic antidepressants such as amitriptyline in oral dosages of 25 to 150 mg each day is partially effective in 40% to 66% of treated patients.[36,37] Regimens consisting of carbamazepine given in dosages of 100 to 200 mg orally three times each day or phenytoin, 100 mg given orally three times each day, appear to be similarly effective. Occasionally a patient will respond to transcutaneous nerve stimulation techniques and cognitive behavioral therapy in the context of a comprehensive pain management program.

Chronic lumbosacral pain is frequently associated with mechanical stress on the spine, with spasticity and weakness. Physical therapy and nonsteroidal anti-inflammatory agents are frequently of benefit. It is important to maintain vigilance for radiculopathy from disc herniation because patients with multiple sclerosis commonly have associated degenerative disc disease.[36]

Painful tonic spasms of the limbs are commonly triggered by tactile stimuli, positional change, decubitus, and urinary tract infections. Antibiotic treatment of obvious or subclinical infections is often effective. The use of baclofen in dosages similar to those required for the management of spasticity is reported to be effective therapy in about two thirds of patients.[36]

Acute Pain

The acute pain syndromes in multiple sclerosis

include trigeminal neuralgia, painful dysesthesia, Lhermitte's sign, or dystonic spasms (vide infra). Trigeminal neuralgia is more commonly bilateral (31% versus 10%) and seen at a younger age (50 versus 63.5 years),[38] but it is otherwise indistinguishable from trigeminal neuralgia occurring in patients who do not have multiple sclerosis. Each of these conditions is generally partially or completely responsive to the administration of carbamazepine, 100 to 200 mg administered orally two or three times each day.[39,40] Phenytoin, 100 to 200 mg two or three times each day,[41] and baclofen, 10 to 30 mg one to three times each day,[42] are similarly effective alternatives, and early experience with gabapentin, 300 to 400 mg three times a day, has been promising. Percutaneous trigeminal rhizotomy may be indicated for medically refractory cases.

Dystonic Spasms

Dystonic spasms or "tonic seizures" consist of paroxysmal tonic posturing that affects one or more extremities and occasionally affects one side of the face. In most patients, dystonic spasms are painful and stereotyped, spreading over the involved area over several seconds and at times crossing the midline. They may last as long as two minutes, be preceded or followed by sensory disturbances, and recur repeatedly. Frequently these spasms are triggered by hyperventilation, movement, or tactile stimulation of the limb. Some patients suggest that alcohol consumption may trigger an event. Dystonic spasms bear no relation to the degree of underlying spasticity and are differentiated from simple flexor spasms by the greater intensity of the pain, longer duration, stereotypy, the possible presence of sensory symptoms, and triggering factors. Dystonic spasms are not epileptic, do not impair consciousness, and generally respond well to the use of carbamazepine or phenytoin orally in dosages of 100 to 200 mg two to three times each day.

Tremor and Ataxia

Tremor and ataxia are among the most debilitating consequences of multiple sclerosis. These disorders are poorly responsive to pharmacotherapy. The use of clonazepam, 0.5 to 2 mg orally one to four times each day,[25] and primidone, 125 to 250 mg orally two to three times each day, is partially effective in a few patients. Occasionally a patient may be partially responsive to the administration of propranolol hydrochloride, 20 to 40 mg orally two to three times a day. Inhaled marijuana is not beneficial,[43] and isolated reports of success with other agents, including isoniazid, carbamazepine, acetazolamide, and glutethimide, have not been confirmed in practice.

REFERENCES

1. LaRocca NG, Kalb RC: Efficacy of rehabilitation for multiple sclerosis. J Neurol Rehabil 1992; 6:147–150

2. Goodkin DE: Role of steroids and immunosuppression and effects of interferon beta-1b in multiple sclerosis, *In* Neurology—From Basics to Bedside [Special Issue]. West J Med 1994; 161:292–298

3. Milligan NM, Newcombe R, Compston DA: A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis—1. Clinical effects. J Neurol Neurosurg Psychiatry 1987; 50:511–516

4. Durelli L, Cocito D, Riccio A, et al: High-dose intravenous methylprednisolone in the treatment of multiple sclerosis: Clinical-immunologic correlations. Neurology 1986; 36:238–243

5. Thompson AJ, Kennard C, Swash M, et al: Relative efficacy of intravenous methylprednisolone and ACTH in the treatment of acute relapse in multiple sclerosis. Neurology 1989; 39:969–971

6. Barnes MP, Bateman DE, Cleland PG, et al: Intravenous methylprednisolone for multiple sclerosis in relapse. J Neurol Neurosurg Psychiatry 1985; 48:157–159

7. Abbruzzese G, Gandolfo C, Loeb C: 'Bolus' methylprednisolone versus ACTH in the treatment of multiple sclerosis. Ital J Neurol Sci 1983; 4:169–172

8. Alam SM, Kyriakides T, Lawden M, Newman PK: Methylprednisolone in multiple sclerosis: A comparison of oral with intravenous therapy at equivalent high dose. J Neurol Neurosurg Psychiatry 1993; 56:1219–1220

9. Troiano RA, Hafstein MP, Zito G, Ruderman MI, Dowling PC, Cook SD: The effect of oral corticosteroid dosage on CT-enhancing multiple sclerosis plaques. J Neurol Sci 1985; 70:67–72

*10. Myers LW: Treatment of multiple sclerosis with ACTH and corticosteroids, *In* Rudick RA, Goodkin DE (Eds): Treatment of Multiple Sclerosis—Trial Design, Results and Future Perspectives. New York, NY, Springer-Verlag, 1992, pp 135–156

11. Barkhof F, Hommes OR, Scheltens P, Valk J: Quantitative MRI changes in gadolinium-DTPA enhancement after high-dose intravenous methylprednisolone in multiple sclerosis. Neurology 1991; 41:1219–1222

12. Daynes RA, Araneo BA: Contrasting effects of glucocorticoids on the capacity of T cells to produce the growth factors interleukin 2 and interleukin 4. Eur J Immunol 1989; 19:2319–2325

13. AyanlarBatuman O, Ferrero AP, Diaz A, Jimenez SA: Regulation of transforming growth factor-β1 gene expression by glucocorticoids in normal human T lymphocytes. J Clin Invest 1991; 88:1574–1580

14. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G: A mechanism for the anti-inflammatory effects of corticosteroids: The glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. Proc Natl Acad Sci USA 1992; 89:9991–9995

15. Beck RW, Cleary PA, Trobe JD, et al: The effect of corticosteroids for acute optic neuritis on the subsequent development of multiple sclerosis—The Optic Neuritis Study Group. N Engl J Med 1993; 329:1764–1769

16. Beck RW: Strategies to delay the onset of clinically definite MS in patients with monosymptomatic presentations of optic neuritis, brainstem syndromes or myelopathy, *In* Goodkin DE, Rudick RA (Eds): Multiple Sclerosis: Advances in Trial Design, Results and Future Perspectives. London, Engl, Springer-Verlag, 1996, pp 201–222

*17. Parziale JR, Akelman E, Herz DA: Spasticity: Pathophysiology and management. Orthopedics 1993; 16:801–811

18. Young RR, Delwaide PJ: Drug therapy: Spasticity (2nd of 2 parts). N Engl J Med 1981; 304:96–99

19. McLean BN: Intrathecal baclofen in severe spasticity. Br J Hosp Med 1993; 49:262–267

20. Betts CD, D'Mellow MT, Fowler CJ: Urinary symptoms and the neurological features of bladder dysfunction in multiple sclerosis. J Neurol Neurosurg Psychiatry 1993; 56:245–250

*21. Fowler CJ, van Kerrebroeck PE, Nordenbo A, Van Poppel H: Treatment of lower urinary tract dysfunction in patients with multiple sclerosis—Committee of the European Study Group of SUDIMS (Sexual and Urological Disorders in Multiple Sclerosis). J Neurol Neurosurg Psychiatry 1992; 55:986–989

22. Webb RJ, Lawson AL, Neal DE: Clean intermittent self-catheterisation in 172 adults. Br J Urol 1990; 65:20–23

23. Hinds JP, Eidelman BH, Wald A: Prevalence of bowel dysfunction in multiple sclerosis: A population survey. Gastroenterology 1990; 98:1538–1542

24. Glick ME, Meshkinpour H, Haldeman S, Bhatia NN, Bradley WE: Colonic dysfunction in multiple sclerosis. Gastroenterology 1982; 83:1002–1007

*25. Rudick RA, Goodkin DE, Ransohoff RM: Pharmacotherapy of multiple sclerosis: Current status. Cleve Clin J Med 1992; 59:267–277

26. Krupp LB, Coyle PK, Douscher C, et al: Fatigue therapy in MS: Results of a double-blind randomized parallel trial of amantadine, pemoline and placebo. Neurology 1995; 45:1956–1961

27. Canadian MS Research Group: A randomised controlled trial of amantadine in fatigue associated with multiple sclerosis. Can J Neurol Sci 1987; 14:273–278

28. Cohen RA, Fisher M: Amantadine treatment of fatigue associated with multiple sclerosis. Arch Neurol 1989; 46:676–680

29. Heitpas J, Hooks ML, Atchison P, Williams Pedretti L, McCormack GL: Degenerative diseases of the central nervous system, *In* Williams Pedretti L (Ed): Occupational Therapy: Practice Skills for Physical Dysfunction. New York, NY, Mosby, 1996, pp 837–842

30. Joffe RT, Lippert GP, Gray TA, Sawa G, Horvath Z: Mood disorder and multiple sclerosis. Arch Neurol 1987; 44:376–378

31. Schiffer RB, Herndon RM, Rudick RA: Treatment of pathologic laughing and weeping with amitriptyline. N Engl J Med 1985; 312:1480–1482

*32. Beatty WW: Cognitive and emotional disturbances in multiple sclerosis. Neurol Clin 1993; 11:189–204

33. Minden SL, Schiffer RB: Affective disorders in multiple sclerosis: Review and recommendations for clinical research. Arch Neurol 1990; 47:98–104

34. Sadovnick AD, Remick RA, Allen J, et al: Depression and multiple sclerosis. Neurology 1996; 46:628–632

35. Sadovnick AD, Eisen K, Ebers GC, Paty DW: Cause of death in patients attending multiple sclerosis clinics. Neurology 1991; 41:1193–1196

*36. Moulin DE, Foley KM, Evers GE: Pain syndromes in multiple sclerosis. Neurology 1988; 38:1830–1834

37. Clifford DB, Trotter JL: Pain in multiple sclerosis. Arch Neurol 1984; 41:1270–1272

38. Brisman R: Trigeminal neuralgia and multiple sclerosis. Arch Neurol 1987; 44:379–381

39. Espir MLE, Millac P: Treatment of paroxysmal disorders in multiple sclerosis with carbamazepine (Tegretol). J Neurol Neurosurg Psychiatry 1970; 33:528–531

40. Sweet WH: The treatment of trigeminal neuralgia (tic douloureux). N Engl J Med 1986; 315:174–177

41. Katusic S, Beard CM, Bergstralh E, Kurland LT: Incidence and clinical features of trigeminal neuralgia, Rochester, Minnesota, 1945–1984. Ann Neurol 1990; 27:89–95

42. Fromm GH, Terrence CF, Chattha AS: Baclofen in the treatment of trigeminal neuralgia: Double-blind study and long-term follow-up. Ann Neurol 1984; 15:240–244

43. Greenberg HS, Werness SAS, Pugh JE, Andrus RO, Anderson DJ, Domino EF: Short-term effects of smoking marijuana on balance in patients with multiple sclerosis and normal volunteers. Clin Pharmacol Ther 1994; 55:324–328

*An asterisk denotes a review of a particular symptom.

This article is one of a series on topics in primary care in which common diagnostic or therapeutic problems encountered in primary care practice are presented. Physicians interested in contributing to the series are encouraged to contact the series' editors.

STEPHEN J. McPHEE, MD
TERRIE MENDELSON, MD
Assistant Editors

EXHIBIT I

Clinical review

Recent advances

Neurology

A J Larner, S F Farmer

Department of Neurology, St Mary's Hospital, London W2 1NY
A J Larner, *senior registrar*
S F Farmer, *consultant*

Correspondence to: S F Farmer, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG
s.farmer@ion.ucl.ac.uk

BMJ 1999;319:362–6

Most neurological problems are dealt with by general practitioners and hospital physicians, not by neurologists.[1] Neurological disorders account for 10%-20% of acute hospital admissions. Around 10% of the adult population consult their general practitioner each year with neurological symptoms, but of these less than 10% are referred to hospital clinics. Developments in the management of neurological disorders are therefore relevant to doctors without specialist neurological training.

Methods

We identified references by regular reading of general medical and neurological journals, from searching the electronic literature (Medline, BIDS), and through discussion with general practitioners and neurological colleagues with specialist interests. The final selection of papers was partly subjective.

Cerebrovascular disease

The international stroke trial and the Chinese acute stroke trial, each concerning around 20 000 patients, examined antithrombotic therapy (aspirin, heparin) given within 48 hours of acute ischaemic stroke.[2,3] Both found aspirin to be associated with about 10 fewer deaths or recurrent strokes in the first 4 weeks for each 1000 patients treated, but with slightly more haemorrhagic strokes. The international stroke trial reported no benefit from subcutaneous heparin (5000 or 12 500 IU twice daily) given with or without aspirin. Hence it was concluded that aspirin should be started as soon as possible after the onset of an acute ischaemic stroke.[2,3] Whether aspirin use is "acute treatment" or simply early secondary prevention remains debatable.

No clinical indicators reliably differentiate ischaemic from haemorrhagic stroke. The recommendation that aspirin be started only after appropriate brain imaging in patients requiring admission to hospital will place a huge burden on acute neurological services (over 100 000 people have a first stroke in England and Wales each year). The issue is still more problematic for thrombolytic therapy (tissue type plasminogen activator, streptokinase, urokinase). An overview of previous trials indicated significant excesses of early and total deaths, and of symptomatic and fatal intracranial haemorrhages, after acute thrombolytic therapy, but a significant reduction in death or dependency in patients randomised to treatment within 3 hours of stroke onset.[4] To identify the small number of patients likely to benefit from thrombolysis, early hospital admission and prompt investigation will be necessary. This may be achieved in dedicated stroke units, which have been shown to produce long term reductions in death, dependency, and need for institutional care.[5]

Carotid artery stenosis is an important predisposing factor for cerebrovascular ischaemic events, the risk increasing with the severity of stenosis and the presence of symptoms. For severe (more than 70% narrowing) symptomatic stenosis, carotid endarterectomy is recommended. For severe symptom free stenosis, optimal management has yet to be defined: a meta-analysis of trials[6] showed only a small absolute benefit from surgery in reducing the odds of ipsilateral stroke; hence the procedure cannot be routinely recommended. For mild to moderate symptomatic stenosis (less than 70% narrowing), antiplatelet therapy with aspirin or dipyridamole, or both, is recommended. Persistent symptoms may necessitate use of other treatments such as ticlopidine (named patient basis only); clopidogrel, which reduces the relative risk for further ischaemic events slightly more than aspirin[7]; or anticoagulation with warfarin.

Epilepsy

Most individuals with newly diagnosed epilepsy enter prolonged seizure remission and have an excellent prognosis, but seizures remain refractory in 20%-30%.[8] Improved evaluation of such patients with magnetic resonance imaging and telemetry may identify the structural and functional abnormalities that give rise to seizures. Up to 75% of patients with refractory partial epilepsy show evidence of abnormalities on magnetic resonance imaging,[9] some of which are amenable to surgery.

Cerebrovascular disease

- Given within 48 hours of ischaemic stroke, aspirin reduces risk of death and recurrent stroke
- Thrombolysis for acute ischaemic stroke is most effective if delivered within 3 hours of stroke onset
- Stroke units reduce death, dependency, and need for institutional care after stroke
- Carotid endarterectomy is currently recommended only for severe and symptomatic carotid stenoses

The first line drugs for epilepsy monotherapy remain carbamazepine and sodium valproate; phenytoin is now less used, and although lamotrigine has a monotherapy licence its place has still to be defined. Several new "add on agents" have been licensed in recent years including vigabatrin, gabapentin, lamotrigine, and topiramate. An overview of trials in patients with refractory partial seizures suggests no major differences between these agents in either efficacy or tolerability.[10] Severe visual field defects have recently been reported after prolonged use of vigabatrin, prompting the development of guidelines for monitoring vision.[11] Vagal stimulation remains an experimental approach to seizure control.[12]

Population based studies show that patients with epilepsy have an increased risk of death compared with age and sex matched controls.[13] Some of these deaths are related to epilepsy itself, for example, as a consequence of accidents, but others are unexplained. The category of "sudden unexpected death in epilepsy" (SUDEP) has recently been introduced to encompass all such deaths, which are more common in individuals with refractory epilepsy (about 1 per 200 patients per year).[13] Many of these deaths may be related to unwitnessed seizures, possibly associated with ventricular fibrillation, asystole, respiratory arrest, or neurologically mediated pulmonary oedema. A proportion of cases of sudden unexpected death in epilepsy may therefore be preventable with improved seizure control.

Epilepsy

- Evaluation of patients with refractory partial epilepsy with magnetic resonance imaging and telemetry may yield diagnostic information in up to 75%
- Second line, "add on," agents for refractory partial seizures show no significant differences in efficacy or tolerability
- Guidelines for monitoring of visual fields in patients receiving long term vigabatrin have been issued
- Sudden unexplained death in epilepsy (SUDEP) may be preventable with better seizure control

Multiple sclerosis

- Inteferon betas reduce relapse rate in relapsing-remitting multiple sclerosis by about one third in patients experiencing two disabling relapses every 2 years, and may have a small effect in delaying disability progression
- Magnetic resonance imaging can help predict which patients with clinically isolated syndromes will progress to multiple sclerosis
- Liaison between primary and secondary healthcare services is essential for the appropriate management of established disability in multiple sclerosis

Multiple sclerosis

Interferon betas (interferon beta-1b, Betaferon; interferon beta-1a, Avonex, Rebif) have been shown to reduce relapse rate in relapsing-remitting (non-progressive) multiple sclerosis by about one third.[14–16] The Association of British Neurologists recommends (guidelines, June 1999) interferon beta be prescribed for ambulant patients with at least two definite relapses in the previous 2 years followed by recovery (may or may not be complete). Whether reduction in relapse rate reduces or prevents later disability is not known; some evidence has been presented in favour.[15 16] The uncertainty reflects the difficulties of treatment trials for multiple sclerosis owing to the variable clinical course of the disease, the validation of disability measurements, and discrepancies between secondary outcome measures such as evidence of changes on magnetic resonance imaging, and clinical behaviour.[17] Interferon beta-1b has been reported to delay progression (for 9-12 months in a study period of 2-3 years) in secondarily progressive multiple sclerosis of moderate severity (minimum walking distance of 20 metres with assistance)[18] and has been licensed for this indication. The Association of British Neurologists has produced guidelines to aid therapeutic decision making in secondary progressive multiple sclerosis, but these may be altered in the light of a recent large study (SPECTRIMS; to date presented only in abstract), which reported no significant effect of interferon beta-1a in delaying disability in secondary progressive multiple sclerosis. Sorting out this complex area, with profound financial implications, will be a high priority for NICE.

Trials are also in progress to ascertain whether interferon betas are useful in primary progressive multiple sclerosis, or in delaying or preventing the onset of disseminated disease after clinically isolated syndromes such as optic neuritis and transverse myelitis. Defining which of these patients will progress to widespread disease may be facilitated by magnetic resonance imaging; clinically silent lesions are predictive of the long term risk of subsequent development of multiple sclerosis.[19]

Other possible treatments for multiple sclerosis include copolymer-1[20] and pulsed intravenous immunoglobulin,[21] which, like interferon betas, reduce the relapse rate. However, the place for these immunomodulatory agents remains to be defined. Intravenous methylprednisolone may hasten recovery from acute relapses but has no effect in the long term. A recent trial suggested intravenous methylprednisolone is no better than equivalent oral doses of methylprednisolone for acute relapses.[22] If so, then switching from intravenous to oral steroids for acute relapses may make considerable savings.

In advanced multiple sclerosis, management of established disability may be the primary role of the neurologist. Bladder dysfunction usually consists of combined detrusor hyperreflexia and incomplete emptying volumes of less than 100 ml of urine remaining in the bladder after micturition are managed with oxybutinin or detrusitol; volumes greater than 100 ml require clean, intermittent, self catheterisation.[23] Sexual dysfunction (erectile failure) may be helped with the phosphodiesterase inhibitor sildenafil citrate (Viagra), or more established agents such as yohimbine or other α adrenoreceptor blockers. Management of limb spasticity requires a multidisciplinary approach ensuring correct posture, prevention of skin ulceration from pressure, management of bladder and bowel dysfunction, as well as pharmacological measures. Tizanidine, an α_2 adrenoreceptor agonist, has recently been licensed in the United Kingdom as an antispastic agent.[24]

Parkinson's disease

The management of idiopathic Parkinson's disease is still centred around the use of levodopa preparations since they produce the most effective relief of

Parkinson's disease

- Accurate diagnosis of parkinsonian syndromes is important for appropriate management
- Use of levodopa sparing agents may be desirable in the treatment of early onset Parkinson's disease
- The late stages of Parkinson's disease necessitate the use of polypharmacy to manage unpredictable response fluctuations to levodopa, which are an almost invariable feature after several years' treatment
- Functional neurosurgery may improve some of the features of Parkinson's disease and treatment complications in selected patients

symptoms in most patients. Distinguishing idiopathic Parkinson's disease from other parkinsonian syndromes (for example, multiple system atrophy, Steele-Richardson-Olszewski syndrome) is crucial as the latter differ in prognosis and management. With long term treatment with levodopa, response fluctuations develop in around 10% of patients with idiopathic Parkinson's disease per year. Delaying the use of levodopa in early disease, particularly in young patients (under 50 years), is therefore desirable.

Levodopa sparing agents that may be used as monotherapy in early Parkinson's disease include amantadine, anticholinergics (particularly if tremor is predominant), selegiline, and dopamine agonists. Bromocriptine alone improves about 50% of patients during the first year of treatment but there is a gradual loss of benefit thereafter, with only 10% responding at 5 years; other dopamine agonists have similar effects. Selegiline delays the need for levodopa by a mean of 8 months. One study found increased mortality in patients taking levodopa in combination with selegilne.[25]

Once levodopa is started it may be several years before response fluctuations develop. These are usually predictable initially, such as end of dose or "wearing off" effects. Strategies to ameliorate these problems include dose fractionation, long acting levodopa preparations (particularly helpful for nocturnal immobility), and addition of dopamine agonists.[26] Three new dopamine agonists are now available: ropinirole, cabergoline, and pramipexole.[27-29] It is not yet known whether these agents confer significant therapeutic advantages over bromocriptine and pergolide as add on therapy. Add on use of catechol-O-methyltransferase inhibitors (tolcapone, entacapone) may increase total "on" time by about 20%,[30] but tolcapone has been withdrawn in the United Kingdom because of hepatotoxicity.

Sudden unpredictable changes between periods of mobility ("on") with severe levodopa induced involuntary movements (dyskinesias) and disabling parkinsonism ("off"), the "on-off phenomenon", present a major management problem. Various strategies may be tried to minimise the severity of dyskinesias[31]—for example, adjusting the timing of levodopa intake, optimising levodopa absorption, and addition of other antiparkinsonian agents such as dopamine agonists and amantadine thus permitting levodopa dose reduction. Increased use of the dopamine agonist apomorphine, given by subcutaneous injection or infusion, may rescue patients from severe sudden off periods, and improve overall mobility.

Renewed interest has been shown in surgery for the symptoms of Parkinson's disease, levodopa induced dyskinesias, and disorders of movement such as tremor and dystonia through the use of stereotactically placed lesions or chronic electrical stimulation with indwelling electrodes.[32] Thalamotomy or thalamic stimulation helps tremor and often rigidity but not akinesia. Unilateral pallidotomy (figure) significantly improves contralateral dyskinesias; bilateral pallidotomy may, in addition, improve parkinsonian symptoms but carries increased risks.[33] Bilateral subthalamic nucleus stimulation[34] or subthalamotomy has produced dramatic improvements in parkinsonism, sufficient to allow large reductions in the levodopa dose and thus improvement in levodopa induced dyskinesias. Appropriate patient selection is a key factor in its successful use, and it will be available only in a few specialist centres.

Dementia

The definition of a new variant of Creutzfeldt-Jakob disease[35] has attracted huge media attention, because it seems to be caused by the same strain of prion protein that causes bovine spongiform encephalopathy, and hence may have been transmitted to humans through contaminated food. However, this uniformly fatal condition remains extremely rare, with only 41 cases reported to date in the United Kingdom.

For Alzheimer's disease, the commonest cause of dementia, two cholinesterase inhibitors, donepezil (Aricept)[36] and rivastigmine (Exelon),[37] are now licensed for the symptomatic treatment of mild to moderate cognitive impairment (minimental state examination score of 10-26). Trials suggest that in some patients these agents improve cognition, global function, and behavioural function, but there are as yet no data as to whether they delay deterioration or improve outcome. Currently it is recommended that these drugs are only commenced on the advice of a specialist. Despite advances in understanding the pathological basis of Alzheimer's disease, centred around amyloid β peptides, this has not resulted in new treatments although many are in development.[38]

Superimposed 3-dimensional positron emission tomogram and magnetic resonance image of patient with idiopathic Parkinson's disease showing hypermetabolism of pallidum(white area), target for unilateral stereotaxic pallidotomy

Dementia, rare disorders

- Cholinesterase inhibitors are of symptomatic benefit in about 20% of patients with mild to moderate Alzheimer's disease
- Some peripheral neuropathies are treatable may show profound benefit with intravenous immunoglobulin treatment
- Intravenous immunglobulin may be helpful in the acute treatment of Guillain-Barré syndrome and myasthenia gravis

Migraine

Since the launch of sumatriptan, several triptans have become available for the treatment of acute migraine.[39] They differ in pharmacodynamic properties such as time of onset and duration of action. Direct comparisons have not been performed and it is not known whether the differences claimed will have significant impact on clinical practice. Preventive treatments include pizotifen and amitriptyline, but the strongest evidence of efficacy is available for β blockers and sodium valproate.[39]

Rare disorders

The glutamate antagonist riluzole (Rilutek) remains the only licensed treatment for motor neurone disease. Risk of death or tracheostomy was lower with 100 mg riluzole than placebo in limb or bulbar onset disease,[40] but it is debatable whether this translates into an improved quality of remaining life.

Intravenous immunoglobulin has been used in many neurological disorders,[21 41] but its place remains to be defined; many of these applications are empirical and the long term effects of intravenous immunoglobulin are unknown. Trials in acute idiopathic neuropathy (Guillain-Barré syndrome) suggest that intravenous immunglobulin is equivalent to plasma exchange in reducing disability at 4 weeks, but that the combination of intravenous immunoglobulin and plasma exchange offers no significant additional advantage.[42] Likewise, in myasthenia gravis, intravenous immunglobulin seems as efficacious as plasma exchange,[43] and it may also be useful in chronic inflammatory demyelinating polyneuropathy in relapse.[44]

Conclusion

In the past 5 years, new treatments have become available for neurological disorders previously considered untreatable (multiple sclerosis, Alzheimer's disease, motor neurone disease). Although the high cost of these treatments has sometimes led to antagonism between purchasers and doctors wanting to prescribe, these small, initial, therapeutic inroads have focused on the broader needs of patients with neurological disorders, engendering specialist clinics, specialist nurses, and fostering liaison with community services and patient interest groups.

We thank Drs DJ Thomas, RJ Butterworth, M Prevett, P Rudge, D Kidd, CAH Fisher, S Fearn, and S Addison, and Professors NP Quinn and MN Rossor for commenting on earlier drafts of the article.

Competing interests: None declared.

1 Association of British Neurologists. *Neurology in the United Kingdom: towards 2000 and beyond*. London: ABN, 1997.
2 International Stroke Trial Collaborative Group. The international stroke trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19 435 patients with acute ischaemic stroke. *Lancet* 1997;349:1569-81.
3 Chinese Acute Stroke Trial Collaborative Group. CAST: randomised placebo-controlled trial of early aspirin use in 20 000 patients with acute ischaemic stroke. *Lancet* 1997;349:1641-9.
4 Wardlaw JM, Warlow CP, Counsell C. Systematic review of evidence on thrombolytic therapy for acute ischaemic stroke. *Lancet* 1997;350:607-14.
5 Stroke Unit Trialists' Collaboration. Collaborative systematic review of the randomised trials of organised inpatient (stroke unit) care after stroke. *BMJ* 1997;314:1151-9.
6 Benavente O, Moher D, Pham B. Carotid endarterectomy for asymptomatic carotid stenosis: a meta-analysis. *BMJ* 1998;317:1477-80.
7 CAPRIE steering committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996;348:1329-39.
8 Cockerell OC, Johnson AJ, Goodridge DMG, Sander JWAS, Shorvon SD. The remission of epilepsy: results from the national general practice study of epilepsy. *Lancet* 1995;346:140-4.
9 Li LM, Fish DR, Sisodiya SM, Shorvon SD, Alsanjari N, Stevens JM. High resolution magnetic resonance imaging in adults with partial or secondary generalised epilepsy attending a tertiary referral unit. *J Neurol Neurosurg Psychiatry* 1995;59:384-7.
10 Marson AG, Kadir ZA, Chadwick DW. New antiepileptic drugs: a systematic review of their efficacy and tolerability. *BMJ* 1996;313:1169-74.
11 Appleton RE. Guideline may help in prescribing vigabatrin. *BMJ* 1998;317:1322.
12 McLachlan RS. Vagus nerve stimulation for intractable epilepsy: a review. *J Clin Neurophysiol* 1997;14:358-68.
13 Nashef L, Sander JWAS, Fish DR, Shorvon SD. Incidence of sudden unexpected death in an adult outpatient cohort with epilepsy at a tertiary referral centre. *J Neurol Neurosurg Psychiatry* 1995;58:462-4.
14 The Interferon Beta Multiple Sclerosis Study Group and the University of British Columbia Multiple Sclerosis/Magnetic Resonance Imaging Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology* 1995;45:1277-85.
15 Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol* 1996;39:285-94.
16 Prevention of Relapses and Disability by Interferon β-1a Subcutaneously in Multiple Sclerosis Study Group (PRISMS). Randomised double-blind placebo-controlled study of interferon β-1a in relapsing/remitting multiple sclerosis. *Lancet* 1998;352:1498-504.
17 Noseworthy JH, Miller DH. Measurement of treatment efficacy and new trial results in multiple sclerosis. *Curr Opin Neurol* 1997;10:201-10.
18 European Study Group on Interferon β-1b in Secondary Progressive Multiple Sclerosis. Placebo-controlled multicentre randomised trial of interferon β-1b in treatment of secondary progressive multiple sclerosis. *Lancet* 1998;352:1491-7.
19 O'Riordan JI, Thompson AJ, Kingsley DPE, MacManus DG, Kendall BE, Rudge P, et al. The prognostic value of brain MRI in clinically isolated syndromes of the central nervous system. A 10-year follow-up. *Brain* 1998;121:495-503.
20 Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. *Neurology* 1995;45:1268-76.
21 Fazekas F, Deisenhammer F, Strasser-Fuchs S, Nahler G, Marnoli B for the Austrian Immunoglobulin in Multiple Sclerosis Study Group. Randomised placebo-controlled trial of monthly intravenous immunoglobulin therapy in relapsing-remitting multiple sclerosis. *Lancet* 1997;349:589-93.
22 Barnes D, Hughes RAC, Morris RW, Wade-Jones O, Brown P, Britton T, et al. Randomised trial of oral and intravenous methylprednisolone in acute relapses of multiple sclerosis. *Lancet* 1997;349:902-6.
23 Fowler CJ. Investigation of the neurogenic bladder. *J Neurol Neurosurg Psychiatry* 1996;60:6-13.
24 UK Tizanidine Trial Group. A double-blind, placebo-controlled trial of tizanidine in the treatment of spasticity caused by multiple sclerosis. *Neurology* 1994;44(suppl 9):70-78S.
25 Lees AJ on behalf of the Parkinson's Disease Research Group of the United Kingdom. Comparison of therapeutic effects and mortality data of levodopa and levodopa combined with selegiline in patients with early, mild Parkinson's disease. *BMJ* 1995;311:1602-7.
26 Nohria V, Partiot A. A review of the efficacy of the dopamine agonists pergolide and bromocriptine in the treatment of Parkinson's disease. *Eur J Neurol* 1997;4:537-43.
27 Adler CH, Sethi KD, Hauser RA, Davis TL, Hammerstad JP, Bertoni J, et al. Ropinirole for the treatment of early Parkinson's disease. *Neurology* 1997;49:393-9.
28 Rinne UK, Bracco F, Chouza C, Dupont E, Gershanik O, Marti Masso JF, et al. Cabergoline in the treatment of early Parkinson's disease: results of the first year of treatment in a double-blind comparison of cabergoline and levodopa. *Neurology* 1997;48:363-8.
29 Lieberman A, Ranhosky A, Korts D. Clinical evaluation of pramipexole in advanced Parkinson's disease: results of a double-blind, placebo-controlled, parallel-group study. *Neurology* 1997;49:162-8.
30 Baas H, Beiske AG, Ghika J, Jackson M, Oertel WH, Poewe W, et al. Catechol-O-methyltransferase inhibition with tolcapone reduces the "wearing off" phenomenon and levodopa requirements in fluctuating parkinsonian patients. *J Neurol Neurosurg Psychiatry* 1997;63:421-8.

31 Stocchi F, Nordera G, Marsden CD. Strategies for treating patients with advanced Parkinson's disease with disastrous fluctuations and dyskinesias. *Clin Neuropharmacol* 1997;20:95-115.
32 Quinn N, Bhatia K. Functional neurosurgery for Parkinson's disease. *BMJ* 1998;316:1259-60.
33 Samuel M, Caputo E, Brooks DJ, Schrag A, Scaravilli T, Branston NM, et al. A study of medial pallidotomy for Parkinson's disease: clinical outcome, MRI location and complications. *Brain* 1998;121:59-75.
34 Limousin P, Pollak P, Benazzouz A, Hoffmann D, Le Bas J-F, Broussolle E, et al. Effect on parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *Lancet* 1995;345:91-5.
35 Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996;347:921-5.
36 Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT and the Donepezil Study Group. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurology* 1998;50:136-45.
37 Corey-Bloom J, Anand R, Veach J for the ENA 713 B352 Study Group. A randomized trial evaluating the efficacy and safety of ENA 713 (rivastigmine tartrate), a new acetylcholinesterase inhibitor, in patients with mild to moderately severe Alzheimer's disease. *Int J Geriatr Psychopharmacol* 1998;1:55-65.
38 Larner AJ, Rossor MN. Alzheimer's disease: towards therapeutic manipulation of the amyloid precursor protein and amyloid β-peptides. *Exp Opin Ther Patents* 1997;7:1115-27.
39 Ferrari MD. Migraine. *Lancet* 1998;351:1043-51.
40 Lacomblez L, Bensimon G, Leigh PN, Guillet P, meininger V for the Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. *Lancet* 1996;347:1425-31.
41 Wills AJ, Unsworth DJ. A practical approach to the use of intravenous immunoglobulin in neurological disease. *Eur Neurol* 1998;39:3-8.
42 Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. Randomised trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barré syndrome. *Lancet* 1997;349: 225-30.
43 Gajdos P, Chevret S, Clair B, Tranchant C, Chastang C for the Myasthenia Gravis Clinical Study Group. Clinical trial of plasma exchange and high-dose intravenous immunoglobulin in myasthenia gravis. *Ann Neurol* 1997;41:789-96.
44 Hahn AF, Bolton CF, Zochodne D, Feasby TE. Intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy. A double-blind, placebo-controlled, cross-over study. *Brain* 1996;119:1067-77.

Lesson of the week

Mercury poisoning after spillage at home from a sphygmomanometer on loan from hospital

A C Rennie, M McGregor-Schuerman, I M Dale, C Robinson, R McWilliam

Be aware of the potential for toxicity of mercury spilled from broken medical equipment

Royal Hospital for Sick Children, Yorkhill NHS Trust, Glasgow G3 8SJ
A C Rennie, *senior registrar*
C Robinson, *staff grade nephrologist*
R McWilliam, *consultant neurologist*
Law Hospital, Carluke, Lanarkshire ML8 5ER
M McGregor-Schuerman, *consultant paediatrician*
Glasgow Occupational Health, 20 Cochrane Street, Glasgow G1 1JA
I M Dale, *occupational hygienist*
Correspondence to: Dr Rennie alisonrennie@compuserve.com

BMJ 1999;319:366–7

When patients are managed at home, they or their carers have to operate medical equipment. This case report highlights important educational and environmental health aspects of issuing hospital equipment for home use, a practice that is likely to become more common in the future. We describe a 9 year old boy who had neurological and renal complications after mercury spillage from a sphygmomanometer three months after it had been provided by the hospital for monitoring blood pressure at home. The family were unaware of the potential risks of mercury exposure before the patient became acutely ill.

Case report

A 9 year old boy presented to his local hospital with a three week history of abdominal pain, constipation, lethargy, limb pain, and unsteadiness. Physical examination showed mild facial weakness, areflexia, ataxia, and impaired sensation and led to a provisional diagnosis of Guillaine-Barré syndrome. The boy's constant restlessness was considered strange, but his mother described him as hyperactive and regarded this behaviour as normal. It was noted, however, that his handwriting and schoolwork had deteriorated over the preceding month.

Features of encephalopathy accompanied by peripheral neuropathy led to a suspicion of heavy metal poisoning. No history of likely exposure to lead could be found; there was no lead piping or paint at home. Further inquiry revealed that the patient's sibling had undergone renal transplantation as a result of nephrotic syndrome, and the family had been provided with a mercury sphygmomanometer for home blood pressure monitoring. Three months before presentation, our patient had dismantled the sphygmomanometer in his bedroom—spilling mercury on his bed and carpet—and had played with it for a day or two before informing his mother. Attempts had been made to dispose of the mercury by vacuuming, and then by flushing it down the toilet.

The suspected diagnosis of mercury poisoning was confirmed by the finding of a serum mercury concentration of 1000 nmol/l (normal reference value <30 nmol/l). The boy was referred to a tertiary paediatric centre for further management. By now he was unable to pick up objects or to feel them in his hand. Physical examination showed that he was ataxic and areflexic and was exhibiting intermittent aggression and a fluctuating level of consciousness. He was started on sodium-(2,3)-dimercaptopropane-(1)-sulphonate (DMPS), a chelating agent which binds mercury and allows it to be excreted via the kidneys. This is given by intravenous infusion in a reducing dose over four days and is followed by oral treatment until the patient's clinical condition and results of laboratory investigations have improved. Our patient was treated for a total of 18 days; his serum mercury concentrations and urinary mercury excretion during treatment are shown in the table.

Other family members were investigated and were also found to have raised serum mercury concentrations, but in none were these high enough to necessitate treatment. Mercury was not detected in the patient's cerebrospinal fluid, but the protein concentration was very high at 1.9 g/l.

The boy developed hypertension. This was refractory to initial treatment and required an

Serum mercury concentrations and urinary mercury excretion in patient with mercury poisoning

Day	Serum mercury (nmol/l)	Urinary mercury excretion (nmol/mmol creatine)
1	500	173
4	285	650
9	256	241
21	160	223
29	83	24

EXHIBIT J

Abstract

Immunology and Cell Biology
Volume 76 Page 104 - February 1998
doi:10.1046/j.1440-1711.1998.00722.x

Volume 76 Issue 1

Treatment of central nervous system inflammation with inhibitors of basement membrane degradation

CHRISTOPHER R PARISH, ELIZABETH J HINDMARSH, MARK R BARTLETT, MARIA A STAYKOVA, WILLIAM B COWDEN & DAVID O WILLENBORG

Currently available anti-inflammatory drugs for the treatment of multiple sclerosis (MS) and other inflammatory diseases are generally inadequate, with disease progression not being arrested by the treatments and undesirable side effects posing problems. In response to these deficiencies our laboratories have, over the past 10 years, been developing novel drugs that interfere with the entry of leucocytes into inflammatory sites by inhibiting their passage through the subendothelial basement membrane (BM). This review initially summarizes evidence supporting the hypothesis that the subendothelial BM is a major barrier to the accumulation of leucocytes in inflammatory sites. An important point that has emerged is that breaching of the BM is probably a cooperative process, involving activation- and cytokine-induced degradative enzymes contributed by leucocytes, endothelial cells and platelets. The review then discusses the properties of three separate classes of anti-inflammatory compounds we have developed, namely sulfated polysaccharides/oligosaccharides, phosphosugars, and castanospermine (CS), which inhibit the passage of leucocytes through BM. Each drug type appears to prevent BM degradation by a different mechanism. Sulfated polysaccharides/oligosaccharides mediate their anti-inflammatory effect by inhibiting the endoglycosidase, heparanase, which plays a key role in the solubilization of BM by invading leucocytes. In fact, our studies have highlighted the heparanase enzyme as a major target for future drug development. Phosphosugars probably inhibit inflammation by displacing lysosomal enzymes, which are involved in BM degradation, from cell surface mannose 6-phosphate receptors. This mechanism of expressing degradative enzymes on the cell surface is particularly evident with activated T lymphocytes. On the other hand, CS interferes with appropriate targeting of lysosomal enzymes involved in BM degradation. For reasons which are still unclear, CS specifically inhibits BM degradation by endothelial cells, which results in a characteristic perivascular arrest of leucocytes in inflammatory sites. Overall, our studies have established that inhibitors of subendothelial BM degradation represent viable anti-inflammatory agents. It is hoped that future work will result in the development of a totally new class of highly effective, subtle and non-toxic anti-inflammatory drugs for the treatment of MS and other inflammatory diseases.

Keywords:
basement membrane
castanospermine
experimental autoimmune encephalomyelitis
heparanase
mannose phosphate receptors
multiple sclerosis
phosphosugars
sulfated oligosaccharides
sulfated polysaccharides

EXHIBIT K

SECTION EDITOR: IRA SHOULSON, MD

Disease-Modifying Drugs for Relapsing-Remitting Multiple Sclerosis and Future Directions for Multiple Sclerosis Therapeutics

Richard A. Rudick, MD

With the development of effective therapies for multiple sclerosis (MS), therapeutic nihilism, which was so prevalent just 10 years ago, has given way to exuberance and optimism. The current mood is understandable because MS is such a devastating disease. Within 10 years of symptom onset, 50% of patients with MS are unable to carry out household and employment responsibilities; within 15 to 20 years, 50% are unable to walk unassisted; and within 25 years, 50% are unable to walk at all. The average annual cost of MS in the United States has been estimated at greater than $6.8 billion, or $34 103 per person.[1] This review summarizes evidence that disease-modifying drugs can significantly improve the course of patients with relapsing-remitting MS (RRMS) and frames key issues relating to the use of current drugs. Major issues confronting experimental MS therapeutics are discussed.

DRUGS FOR RRMS

Recombinant interferon beta-1b (IFN-β-1b) (Betaseron; Berlex Laboratories Inc, Wayne, NJ), recombinant interferon beta-1a (IFN-β-1a) (Avonex; Biogen Inc, Cambridge, Mass), and glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel) have been approved by the US Food and Drug Administration for patients with RRMS (**Table 1**). These 3 drug therapies were tested in separate multicenter, placebo-controlled, double-masked clinical trials. Key elements of the studies leading to their regulatory approval are summarized in **Table 2**.

Interferon beta-1b therapy was tested in 372 patients at a dosage of 8 million IU (MIU) (250 µg) or 1.6 MIU (50 µg) by subcutaneous injection every other day for up to 5 years, compared with placebo. The primary outcome measure was the drug therapy effect on the relapse rate. Treatment with the higher dosage reduced the relapse rate by 33%, increased the proportion of relapse-free patients from 16% to 31%, and reduced by 2-fold the number of patients having moderate or severe relapses.[14] Beneficial effects were maintained for patients who elected to remain in the blinded trial for up to 5 years.[15] There was a statistically nonsignificant trend ($P = .16$) suggesting that patients in the 8-MIU dosage arm were less likely to experience a worsening by at least 1.0 point from the baseline score on the Expanded Disability Status Scale (EDSS)[16] sustained for at least 3 months.

Interferon beta-1a therapy was tested in 301 patients who were given weekly intramuscular injections (6 MIU [30 µg]) or placebo for up to 2 years.[4,5] The primary outcome measure was the time to the onset of sustained disability progression, which was defined as deterioration from baseline by at least 1.0 point on the EDSS persisting for at least 6 months. Treatment with IFN-β-1a resulted in a significantly lower probability of sustained disability progression,[5] and significantly fewer patients treated with IFN-β-1a therapy became severely disabled, defined as at least 6 months of sustained worsening to an EDSS score of 4.0 or 6.0.[17] Patients with an EDSS score of 6.0 require assistance to walk, and their disease course has usually evolved into secondary progressive MS (SPMS). This finding suggests that IFN-β therapy can prevent or delay transition

From the Mellen Center for Multiple Sclerosis Treatment and Research, Department of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio.

Table 1. Drugs for Relapsing-Remitting Multiple Sclerosis (MS) Approved by the US Food and Drug Administration*

	Drug		
	IFN-β-1a (Avonex, Rebif)	IFN-β-1b (Betaseron)	Glatiramer Acetate (Copaxone)
Description	Recombinant IFN-β; glycosylated, amino acid identical to native protein	Recombinant IFN-β; nonglycosylated, serine for cysteine substitution	Random polymer of basic amino acids
Possible mechanisms	Immunomodulatory; inhibits cell migration and cell-mediated inflammation; antiviral effects	Immunomodulatory; inhibits cell migration and cell-mediated inflammation; antiviral effects	Inhibits T-cell recognition of myelin antigens; induces myelin-reactive regulatory cells

**At the time of this review, IFN-β-1a (Rebif) was approved for use in Canada and Europe, and applications were pending in the United States and Australia (G. Francis, MD, oral communication, August 1998). Relapsing-remitting MS refers to the patient with discrete episodes of neurologic deterioration separated by periods of recovery and clinical stability[2]; MS begins in this fashion in approximately 85% of patients. More than 50% of patients enter a stage of continuous neurologic deterioration, termed* secondary progressive MS, *commonly 10 to 20 years after the initial MS symptoms. Therapeutic options have advanced more rapidly for relapsing-remitting MS than for secondary progressive MS (reviewed by Rudick et al[3]). IFN-β indicates interferon beta.*

Table 2. Phase 3 Controlled Clinical Trials in Patients With Relapsing-Remitting Multiple Sclerosis*

	IFN-β-1a (Avonex)	IFN-β-1b (Betaseron)	Glatiramer Acetate (Copaxone)
Trials	Multiple Sclerosis Collaborative Research Group[4,5]	IFNB Multiple Sclerosis Study Group and University of British Columbia MS/MRI Analysis Group[6-8]	Copolymer 1 Multiple Sclerosis Study Group[9,10]
No. of patients			
Placebo	143	123	126
Active treatment	158	125 (1.6 MIU) 124 (8 MIU)	125
EDSS score range†	1.0-3.5	0-5.5	0-5.0
Minimum relapse rate (No. of patients)	0.67 (2 in 3 y)	1.0 (2 in 2 y)	1.0 (2 in 2 y)
Age range, y	16-54	18-50	18-45
Dosage	6 MIU (30 μg) IM weekly	8 MIU (250 μg) or 1.6 MIU (50 μg) SC QOD	20 mg SC QD
Primary outcome measure	Sustained disability	Relapse rate	Relapse rate
Primary result	37% Reduction in disability progression	33% Reduction in relapse rate (8 MIU [250 μg] vs placebo)	29% Reduction in relapse rate
Adverse events	Flulike symptoms (first 2-3 mo)	Flulike symptoms (first 2-3 mo), skin reactions (occasionally severe)	Mild skin reactions; rare systemic reaction with flushing, sweating, and palpitations
NAB	Phase 3 study: 14% NAB after 12 mo, 22% after 24 mo (Jacobs et al[5]) Open-label study: 7% NAB after 12-18 mo; decreased in vivo response to injections (Rudick et al[12])	Phase 3 study: 38% NAB after 24 mo; decreased clinical efficacy (IFNB Multiple Sclerosis Study Group and University of British Columbia MS/MRI Analysis Group[11]) Open-label study: 35% NAB after 12-18 mo (Rudick et al[12])	100% Binding antibodies; no known method to determine significance of binding antibodies

**A multicenter phase 3 placebo-controlled trial of IFN-β-1a (Rebif) was recently completed.[13] The study randomized 560 patients to placebo, 6-MIU (22-μg) dosage of IFN-β-1a, or 12-MIU (44-μg) dosage of IFN-β-1a administered 3 times weekly subcutaneously (SC). There was a 37% reduction in the relapse number after 1 year and a 33% reduction after 2 years at the higher dosage compared with placebo. There were significant beneficial effects on magnetic resonance imaging (MRI) parameters in both dosage arms compared with placebo, and the MRI benefits were greater in the high-dosage compared with the low-dosage group. IFN-β indicates interferon beta; MS, multiple sclerosis; IM, intramuscularly; QOD, every other day; QD, every day; EDSS, Expanded Disability Status Scale; and NAB, neutralizing antibodies.*

†Scale, 0-10.

from RRMS to SPMS in some patients. Treatment with IFN-β-1a significantly reduced the relapse rate by 32% in the cohort of patients treated for 2 years and by 18% in all patients regardless of the time of participation in the study.[5]

Both forms of IFN-β therapy had beneficial effects on the disease process as measured by cranial magnetic resonance imaging (MRI) scans. Interferon beta-1b therapy resulted in significantly fewer new or enlarging T_2-weighted lesions in 52 patients who underwent MRI scan at 1 of the clinical sites every 6 weeks, and IFN-β-1b therapy resulted in significantly less annual accumulation of T_2-weighted lesions in the entire study group.[6] In a separate study, IFN-β-1b therapy reduced the frequency of brain lesions that were enhanced on MRI with gadolinium.[18] In the phase 3 trial, IFN-β-1a therapy sig-

nificantly reduced the number of gadolinium-enhanced MRI brain lesions after 1 and 2 years of treatment and decreased the number of new and enlarging T_2-weighted lesions after 1 and 2 years.[19] These studies indicate that IFN-β therapy inhibits new brain lesion formation. The prominent effect on gadolinium-enhanced MRI lesions suggests that IFN-β therapy reduces brain inflammation. This conclusion was supported by the finding that IFN-β-1a therapy lessened cerebrospinal fluid pleocytosis.[20]

Both IFN-β preparations cause transient flulike symptoms. Headache, myalgia, fever, malaise, and occasionally increased MS symptoms commonly last 24 to 48 hours after each injection; the severity of these symptoms typically lessens after 6 to 12 weeks of therapy. Interferon beta-1b therapy causes redness and swelling at the injection site and skin necrosis in 5% of patients. In the phase 3 clinical trials, neutralizing antibodies to IFN-β-1b were observed in 38% of patients[11] and antibodies to IFN-β-1a in 22% of patients after 2 years of treatment.[5] The presence of neutralizing activity in the IFN-β-1b study was associated with reduced clinical and MRI efficacy. In an open-label study, a single biological assay was used to determine titers of neutralizing antibodies in patients treated clinically with IFN-β-1b or IFN-β-1a.[12] After 12 to 18 months of treatment, neutralizing antibodies were observed in 35% of the patients treated with IFN-β-1b and 7% of patients treated with IFN-β-1a, suggesting that IFN-β-1b therapy is more immunogenic. This may be because of known molecular differences between the preparations. Additionally, the dosage, route, or timing of administration may affect immunogenicity. The presence of neutralizing antibodies in the open-label study was associated with significantly blunted in vivo induction of β_2-microglobulin and neopterin following IFN-β-1a injections.[12] This indicates that patients receiving IFN-β preparations who develop neutralizing antibodies have significantly blunted in vivo biological responses to IFN-β injections at the time they are antibody-positive.

Interferon β induces the expression of many genes, so the mechanisms of action in MS are probably complex.[21] Putative mechanisms include (1) inhibition of autoreactive T cells[22]; (2) inhibition of major histocompatibility complex class II expression,[23] with reduced antigen presentation within the central nervous system; (3) inhibition of metalloprotineases[24,25] or altered expression of cell-associated adhesion molecules,[26] leading to reduced cellular migration into the central nervous system; and (4) induction of immunosuppressive cytokines[27] and inhibition of proinflammatory cytokines,[28] leading to resolution of the inflammatory process.

Glatiramer acetate (Copaxone) is a polypeptide consisting of a random arrangement of 4 basic amino acids. The drug is thought to mimic myelin basic protein and is postulated to induce myelin-specific suppressor T cells and to inhibit myelin-specific effector T cells.[9] Glatiramer acetate therapy was tested in 251 patients who were given daily subcutaneous injections (20 mg or placebo)[9] for 2 years. The primary outcome measure was the effect of the drug on the relapse rate. In the original 2-year study, glatiramer therapy reduced the relapse rate by 29%. At the end of 2 years of therapy, patients were offered entry to an extension study that was continued in a double-masked manner for about 1 year. A large majority of patients continued in the extension study, and the beneficial effect on the relapse rate was maintained.[10] No significant effect was observed on sustained changes in EDSS scores, either in the original study or the extension study. Glatiramer therapy was well tolerated by the patients. Mild swelling and redness occurred at each injection site and 15% of the patients experienced brief episodes of flushing, chest tightness, palpitations, dyspnea, and anxiety.

Magnetic resonance imaging scans were not included as part of the glatiramer phase 3 study, but 27 cases had serial MRI scans at 1 of the sites.[29] There was a trend toward reduced gadolinium-enhanced MRI lesions for patients receiving glatiramer therapy, but no statistically significant benefits were noted on any MRI parameter (J. A. Cohen, MD, oral communication, October 1998). A similar trend toward reduced gadolinium-enhanced MRI lesions was found in a small study of 10 patients receiving glatiramer therapy.[30] A placebo-controlled study was recently completed and demonstrated a significant 30% reduction in new MRI lesions with glatiramer (Copaxone) therapy (G. Comi, MD, oral communication, April 1999).

CONTEMPORARY ISSUES ABOUT APPROVED MS DRUGS

Which of the Available Drugs Is Most Efficacious?

The phase 3 studies convincingly demonstrated that each drug is partially effective, but precise comparisons are problematic. The studies were done by different investigator groups using separate primary outcome measures in separate patient populations. Traditional clinical outcome measures, such as relapse rate and EDSS scores, are imprecise and not adequately standardized to allow direct comparisons among studies. Therefore, efficacy comparisons are based on expert opinions rather than definitive comparison studies. Neurologists who recommend IFN-β therapy as the first-line drug therapy argue that the overall weight of evidence favors IFN-β over glatiramer therapy. Three separate study groups independently demonstrated the efficacy of IFN-β therapy in large, well-controlled, double-blind clinical trials, while a single phase 3 study evaluated glatiramer therapy. Furthermore, IFN-β therapy has been shown to favorably affect disease parameters visualized by MRI and has been shown to decrease cerebrospinal fluid cellularity. Data on the effects of glatiramer therapy on biological correlates of the MS disease process are currently limited. Proponents of IFN-β-1b therapy argue that (1) demonstrated beneficial effect on T_2-weighted lesion accrual after 2 years was greater with IFN-β-1b therapy than with IFN-β-1a therapy; (2) IFN-β-1b therapy is given at a higher weekly dosage, which may be better; and (3) IFN-β-1b therapy was associated with a larger reduction in the relapse rate than was IFN-β-1a therapy. Proponents of IFN-β-1a therapy argue that (1) results showed reduced disability progression that was not evident in the IFN-β-1b

therapy study; (2) injection site reactions that are commonly caused by IFN-β-1b therapy are not observed with IFN-β-1a therapy; (3) IFN-β-1a therapy is less immunogenic than IFN-β-1b therapy, resulting in greater biological response over time; and (4) patients prefer the weekly dosage schedule and favorable side-effect profile of IFN-β-1a therapy. Proponents of glatiramer therapy argue that (1) the drug is better tolerated than IFN-β preparations and (2) glatiramer therapy circumvents the problem of IFN-β–neutralizing antibodies observed in a proportion of IFN-β therapy recipients who take either preparation. Since there are no studies comparing the efficacy of the available drugs within a single study, the question of relative efficacy is considered unresolved.

When Should Therapy Be Initiated, and What Is the Optimal Duration of Therapy?

There is a growing consensus that disease-modifying therapy should be initiated early in the course of MS before irreversible disability has occurred. The rationale for early therapy includes (1) concerns that the immunologic process leading to tissue injury becomes more complex as time passes and may be more difficult to control with immunosuppressive therapy,[31,32] (2) increasing awareness that the inflammatory process is active in many patients with RRMS during periods of clinical remission,[33-35] and (3) concern that the inflammatory process results in irreversible axonal injury[36] that accumulates over time during the relapsing-remitting stage of MS. These considerations imply that disease-modifying therapy should be started when MS is definitively diagnosed because the patient is at risk for subsequent disability progression. Trials of IFN-β-1a therapy beginning with the first MS symptom are under way and may help to clarify this issue.

Identifying patients at higher risk for progressive MS for early therapy is an alternative to treating all patients at the time of diagnosis. Unfortunately, clinical features are only weak predictors of subsequent disease severity, and their value for assigning prognosis to individual patients is limited. Disease severity as measured by cranial MRI scans at the time of onset of first symptoms has been shown to predict MRI and clinical disease progression.[37] This implies that patients with minimal disease detected by MRI scans could be evaluated with follow-up MRI scans to determine the need for disease-modifying therapy. Identifying prognostic factors early in the course of MS is an important goal of future MS research.

The optimal duration of therapy for MS has not been determined. For patients doing well, therapy should be continued, since a study of IFN-alfa-2a therapy showed increased disease activity when therapy was discontinued after 6 months.[38] Studies are needed in which patients are randomly assigned to continue or stop therapy and then are carefully evaluated under double-masked conditions.

Different disease-modifying therapies should be considered for patients whose condition is deteriorating,[39] particularly patients receiving IFN-β therapy with neutralizing antibodies that persist. Standardized methods for evaluating patients receiving disease therapy are needed, including definitions for those patients who do not respond to treatment.

Should a Patient Receiving One of the Current Drug Therapies Be Evaluated With Periodic MRI Scans?

The poor relationship between clinical relapses and the severity of brain inflammation implies that more accurate and sensitive markers of the pathologic process in RRMS will be required. Periodic cranial MRI scans may be useful in estimating MS disease activity and progression in some patients, to determine the need for disease-modifying therapy in patients with clinically benign disease, and to evaluate the response to disease-modifying therapy. Studies are needed to precisely define the methods and frequency for using MRI to monitor patients receiving disease-monitoring therapy.

Should Patients Receiving IFN-β Therapy Routinely Have Tests for Neutralizing Antibodies?

Patients who continue to have clinical disease activity despite IFN-β therapy should have their serum levels tested for neutralizing antibodies.[40] If the assay is negative, IFN-β therapy could be continued and the addition of other medications, such as azathioprine or methylprednisolone, could be considered. There is controversy about whether patients receiving IFN-β therapy who are doing well should be routinely tested for neutralizing antibodies. Advocates argue that high levels of neutralizing antibodies block in vivo IFN-β biological responses and that it is not possible to rule out ongoing brain inflammation based only on the clinical symptoms. Further studies on the use of neutralizing antibody tests in clinical practice are needed.

Should Patients With SPMS Be Treated With Available Drugs?

A multicenter, placebo-controlled study of IFN-β-1b was completed in Europe recently. The study found a significantly longer time to sustained worsening in EDSS scores, reduced relapse frequency, and beneficial effects observed by serial MRI scans in patients who received IFN-β-1b therapy).[41] Separate studies of IFN-β-1b and IFN-β-1a therapy are ongoing in populations of patients with SPMS. In the near future, there will be a great deal of data on which to judge the magnitude of clinical benefit of IFN-β treatment in patients with SPMS.

What Are the Long-term Benefits and Risks of Current MS Drug Therapies, and Do the Long-term Benefits Justify the Cost of the Drugs?

Long-term benefits of the current drug therapies can only be surmised from existing studies because clinical trials run 3 to 5 years, while the disease course of MS unfolds over decades. Clinical trials provide information on only a limited part of the overall disease course. Lengthy placebo-controlled studies are impractical because patients whose condition is deteriorating withdraw from them, making the studies less informative. Lengthy open-label studies do not provide definitive evidence about the efficacy of MS treatment, since patients who are doing well elect to continue receiving drug therapy, while patients whose condition is deteriorating stop drug therapy

to try something else. This results in observer bias favoring long-term efficacy.

Despite their limitations, the studies suggest that available disease therapies are likely to have a beneficial effect on long-term disability, and this might translate into cost-effective treatment. The current cost of the drugs is $8000 to $10 000 per patient annually, which represents approximately 25% of the estimated per-patient annual cost attributed to MS.[1] Long-term cost-benefit analyses are needed.

THE FUTURE OF CONTROLLED CLINICAL TRIALS FOR MS THERAPY

Are Placebo-Controlled Trials Justified?

Placebo-controlled trials for RRMS therapy are now impractical in regions of the world where effective disease-modifying agents are readily available. Furthermore, placebo-controlled trials for RRMS therapy are ethically questionable because of convincing evidence for meaningful, albeit partial, therapeutic benefits. The role of placebo-controlled trials is less clear in patients with SPMS, and this issue can be expected to change, as it has for patients with RRMS, with the emergence of effective therapies. A published study demonstrated statistically significant but clinically modest benefits of low-dose oral methotrexate therapy for patients with chronic progressive MS,[42] and recently completed studies have demonstrated the efficacy of IFN-β-1b therapy and mitoxantrone therapy for patients with SPMS. As results from these and other studies are published, placebo-controlled studies for patients with SPMS will become less practical and more ethically questionable. Since no therapy has demonstrated any benefit for primary progressive MS, placebo-controlled studies for this disease category are well justified.

Can We Improve Clinical Outcome Measures for Future Trials?

An international consensus conference on MS outcome measures pointed out limitations of traditional scales for MS clinical trials and indicated the need for new assessment systems that are multidimensional, quantitative, and include evaluation of cognition.[43] Based on the report from this conference, the National Multiple Sclerosis Society appointed a task force to recommend improved clinical outcome measures. The task force recommended functional composites consisting of simple quantitative tests of neurologic function.[44,45] A 3-part composite that was recommended[46] is currently being tested as an outcome measure in therapeutic trials. It remains to be seen whether quantitative functional composites will prove to be advantageous compared with traditional measures, such as the relapse rate and the EDSS score.

What Is the Relative Role of MRI Compared With Clinical Measures in MS Therapy Trials?

The relationship between MRI abnormalities and clinical disease activity in patients with RRMS is weak. The rate of detection of new gadolinium-enhanced MRI brain lesions is 5 to 10 times higher than the rate of clinical relapses,[33,35] indicating that most new MRI lesions are clinically silent. Similarly, the relationship between the volume of hyperintense T_2-weighted lesions and the EDSS score is also weak.[23] However, it has been demonstrated that patients with RRMS have measurable amounts of ongoing cerebral atrophy,[47] which is also poorly reflected in traditional clinical measures. These findings raise the possibility that in the relapsing-remitting stage of MS, the disease process is subclinical to a substantial degree. The principal concern in this regard is that disability progression occurs only after a threshold of irreversible tissue injury has been surpassed.[36,48] This concept provides a rationale for using MRI measures as outcomes in clinical trials, particularly for patients with RRMS, in whom the neurologic outcomes are imprecise and insensitive to the underlying pathology. Studies are needed to validate traditional and newer MRI markers, such as brain and spinal cord atrophy, as primary outcome measures.

Can We Design Methods to Reliably Test MS Drug Therapies in Combination?

With the advent of partially effective therapies, active arm comparison studies will be needed to make further progress in the field of MS drug therapy. To date, however, no studies have been reported in which drug therapies were tested in combination. Designs for such studies must be developed, and increasingly sensitive and precise outcome measures will be required to achieve practical sample sizes.

Can Therapeutic Interventions Be Rationally Designed to Target Specific Pathogenic Mechanisms?

Most completed and ongoing clinical trials are based on the concept that MS is caused by autoreactive T cells that initiate injury to myelin in the central nervous system. Interventions range from highly specific inhibition of the trimolecular complex to more global forms of immunosuppression. However, recent histopathologic studies[49] suggest that the pathologic characteristics vary significantly among individual patients, raising the possibility that therapy may need to be individualized. Additionally, data indicate that axons and myelin are targets of the pathologic process, providing a rationale for neuroprotective or neurotrophic factors in future clinical trials. Ultimately, improved understanding of pathogenic mechanisms will be needed for selecting rational interventions.

Accepted for publication November 23, 1998.

Presented at the 13th Congress of the European Committee for Treatment and Research in Multiple Sclerosis, Istanbul, Turkey, November 1997.

The author appreciates careful review of the manuscript and helpful suggestions by Jeffrey A. Cohen, MD, and Richard M. Ransohoff, MD.

This review covers articles that were published through November 23, 1998.

Corresponding author: Richard A. Rudick, MD, Department of Neurology, The Mellen Center for Multiple Sclerosis Treatment and Research, U10, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44106 (e-mail: rudikr@cesmtp.ccf.org).

REFERENCES

1. Whetten-Goldstein KFA, Sloan L, Goldstein B, Kulas B. A comprehensive assessment of cost and compensation: the case of multiple sclerosis. *Mult Scler.* 1998; 4:419-425.
2. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology.* 1996;46:907-911.
3. Rudick RA, Cohen JA, Weinstock-Guttman B, Kinkel RP, Ransohoff RM. Management of multiple sclerosis. *N Engl J Med.* 1997;337:1604-1611.
4. Jacobs L, Cookfair DL, Rudick RA, et al, for the Multiple Sclerosis Collaborative Research Group. A phase III trial of intramuscular recombinant interferon beta as treatment for exacerbating-remitting multiple sclerosis: design and conduct of study and baseline characteristics of patients. *Mult Scler.* 1995;1:118-135.
5. Jacobs LD, Cookfair DL, Rudick RA, et al, for the Multiple Sclerosis Collaborative Research Group. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. *Ann Neurol.* 1996;39:285-294.
6. Paty DW, Li DK, for the University of British Columbia MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis, II: MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology.* 1993;43:662-667.
7. The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology.* 1995;45:1277-1285.
8. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis, I: clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology.* 1993;43:656-661.
9. Johnson KP, Brooks BR, Cohen JA, et al, for the Copolymer 1 Multiple Sclerosis Study Group. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. *Neurology.* 1995;45:1268-1276.
10. Johnson KP, Brooks BR, Cohen JA, et al, for the Copolymer 1 Multiple Sclerosis Study Group. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology.* 1998;50:701-708.
11. The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. *Neurology.* 1996;47:889-894.
12. Rudick RA, Simonian NA, Alam JA, et al, for the Multiple Sclerosis Collaborative Research Group. Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis. *Neurology.* 1998;50:1266-1272.
13. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. *Lancet.* 1998;352:1498-1504.
14. The IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis, I: clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology.* 1993;43:655-661.
15. The IFNB Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology.* 1995;45:1277-1285.
16. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* 1983;33:1444-1452.
17. Rudick RA, Goodkin DE, Jacobs LD, et al, for the Multiple Sclerosis Collaborative Research Group. Impact of interferon beta-1a on neurologic disability in relapsing multiple sclerosis. *Neurology.* 1997;49:358-363.
18. Stone LA, Frank JA, Albert PS, et al. The effect of interferon-beta on blood-brain barrier disruptions demonstrated by contrast-enhanced magnetic resonance imaging in relapsing-remitting multiple sclerosis. *Ann Neurol.* 1995;37:611-619.
19. Simon JH, Jacobs LD, Campion M, et al, for the Multiple Sclerosis Collaborative Research Group. Magnetic resonance studies of intramuscular interferon beta-1a for relapsing multiple sclerosis. *Ann Neurol.* 1998;43:79-87.
20. Rudick RA, Cookfair DL, Simonian N, et al. Cerebrospinal fluid abnormalities in a phase III trial of Avonex (IFNβ-1a) for relapsing multiple sclerosis. *J Neuroimmunol.* 1999;93:8-14.
21. Yong VW, Chabot S, Stuve O, Williams G. Interferon beta in the treatment of multiple sclerosis: mechanisms of action. *Neurology.* 1998;51:682-689.
22. Rep MHG, Hintzen RQ, Polman CH, van Lier RAW. Recombinant interferon-beta blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. *J Neuroimmunol.* 1996;67:111-118.
23. Lu HT, Riley JL, Babcock GT, et al. Interferon (IFN) beta acts downstream of IFN-gamma-induced class II transactivator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kd DNA-binding protein, ISGF3-gamma. *J Exp Med.* 1995;182:1517-1525.
24. Leppert D, Waubant E, Burk MR, Oksenbert JR, Hauser SL. Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. *Ann Neurol.* 1996;40: 846-852.
25. Stuve O, Dooley NP, Uhm JH, et al. Interferon beta-1b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9. *Ann Neurol.* 1996; 40:853-863.
26. Calabresi PA, Tranquill LR, Dambrosia JM, et al. Increases in soluble VCAM-1 correlate with a decrease in MRI lesions in multiple sclerosis treated with interferon beta-1b. *Ann Neurol.* 1997;41:669-674.
27. Rudick RA, Ransohoff RM, Lee JC, et al. In vivo effects of interferon beta-1a on immunosuppressive cytokines in multiple sclerosis. *Neurology.* 1998;50:1294-1300.
28. Noronha A, Toscas A, Jensen MA. Interferon beta decreases T cell activation and interferon gamma production in multiple sclerosis. *J Neuroimmunol.* 1993;46: 145-153.
29. Cohen JA, Grossman RI, Udupa JK, et al. Assessment of the efficacy of copolymer-1 in the treatment of multiple sclerosis by quantitative MRI [abstract]. *Neurology.* 1995;45:A418.
30. Mancardi GL, Sardanelli F, Parodi RC, et al. Effect of copolymer-1 on serial gadolinium-enhanced MRI in relapsing remitting multiple sclerosis. *Neurology.* 1998; 50:1127-1133.
31. Yu M, Johnson JM, Tuohy VK. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. *J Exp Med.* 1996;183:1777-1788.
32. Tuohy V, Yu M, Weinstock-Guttman B, Kinkel R. Diversity and plasticity of self recognition during the development of multiple sclerosis. *J Clin Invest.* 1997; 99:1682-1690.
33. Simon JH. Contrast-enhanced MR imaging in the evaluation of treatment response and prediction of outcome in multiple sclerosis. *J Magn Reson.* 1997; 7:29-37.
34. McFarland HF, Stone LA, Calabresi PA, Maloni H, Bash CN, Frank JA. MRI studies of multiple sclerosis: implications for the natural history of the disease and for monitoring effectiveness of experimental therapies. *Mult Scler.* 1996;2:198-205.
35. McFarland HF, Frank JA, Albert PS, et al. Using gadolinium-enhanced magnetic resonance imaging lesions to monitor disease activity in multiple sclerosis. *Ann Neurol.* 1992;32:758-766.
36. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med.* 1998;338:278-285.
37. Filippi M, Horsfield MA, Morrissey SP, et al. Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. *Neurology.* 1994;44:635-641.
38. Durelli L, Bongioanni MR, Ferrero B, et al. Interferon alpha-2a treatment of relapsing-remitting multiple sclerosis: disease activity resumes after stopping treatment. *Neurology.* 1996;47:123-129.
39. Report of the Quality Standards Subcommittee of the American Academy of Neurology. Practice advisory on selection of patients with multiple sclerosis for treatment with Betaseron. *Neurology.* 1994;44:1537-1540.
40. Paty DW, Goodkin D, Thompson A, Rice G. Guidelines for physicians with patients on IFNβ-1b: the use of an assay for neutralizing antibodies. *Neurology.* 1996;47:865-866.
41. European Study Group on interferon beta-1b in secondary progressive MS. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. *Lancet.* 1998;352:1491-1497.
42. Goodkin D, Rudick R, Medendorp S, Greene T. Low-dose (7.5 mg) oral methotrexate for chronic progressive multiple sclerosis: design of a randomized, placebo-controlled trial with sample-size benefits from a composite outcome variable including preliminary data on toxicity. *J Online Curr Clin Trials.* September 25, 1992; document 19. Online journal no longer exists.
43. Whitaker JN, McFarland HF, Rudge P, Reingold SC. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. *Mult Scler.* 1995;1:37-47.
44. Rudick R, Antel J, Confavreux C, et al. Clinical outcomes assessment in multiple sclerosis. *Ann Neurol.* 1996;40:469-479.
45. Rudick R, Antel J, Confavreux C, et al. Recommendations from the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force. *Ann Neurol.* 1997;42:379-382.
46. Cutter GR, Baier ML, Rudick RA, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain.* 1999;122:871-882.
47. Simon JH, Jacobs LD, Campion M, Rudick RA. A longitudinal study of brain atrophy in relapsing MS. *Neurology.* 1999;53:139-148.
48. Rudick RA, Goodman AD, Herndon RM, Panitch HS. Selecting relapsing remitting MS patients for treatment: the case for early treatment. *J Neuroimmunol.* In press.
49. Lucchinetti C, Bruck W, Rodriquez M, Lassmann H. Distinct pattern of multiple sclerosis pathology indicates heterogeneity in pathogenesis. *Brain Pathol.* 1996; 6:259-274.

EXHIBIT L

REVIEW ARTICLE

8.1

Parkinson's disease

C. D. MARSDEN

The recent history of Parkinson's disease has been marked by momentous discoveries. Before the 1960s, the clinical features and basic neuropathology of the disorder had been established, anticholinergic drugs and stereotaxic surgery were popular, but the illness progressed relentlessly and was a cause of miserable disability.[1] The only clues to the cause of Parkinson's disease were its increasing incidence with age, the selective changes involving pigmented brainstem neurons (especially those in substantia nigra pars compacta), and the epidemic of encephalitis lethargica which was followed by post-encephalitic parkinsonism.

The discovery of selective striatal dopamine deficiency in the parkinsonian brain in the early 1960s[2,3] changed everything. The introduction of antipsychotic drugs to control schizophrenia in the 1950s had led to the appearance of drug-induced parkinsonism. How did agents such as reserpine and the phenothiazines produce an akinetic-rigid syndrome indistinguishable from idiopathic Parkinson's disease? Reserpine was found to deplete the brain of monoamines, including noradrenaline, serotonin, and the newly discovered dopamine. Dopamine was found to be concentrated within the basal ganglia, particularly in the striatum (putamen and caudate nucleus), and the dopaminergic nigrostriatal pathway was identified. After the remarkable finding that striatal dopamine content was reduced by 80% or more in the brains of people with Parkinson's disease because of destruction of the pigmented nigral neurons,[3,4] the disease was treated with levodopa to restore striatal dopamine levels, but initial attempts with small intravenous doses gave inconsistent responses.

In the late 1960s and early 1970s high dose oral levodopa therapy was established as the most effective therapy ever found for Parkinson's disease.[5-7] In particular, the most disabling symptoms of the illness, akinesia and bradykinesia, which had shown little improvement with anticholinergics and stereotaxic surgery, responded very well to levodopa. Seriously disabled parkinsonian patients, previously chair-bound or bed-bound by their illness, became mobile with this drug; most patients responded, irrespective of their disability. Parkinson's disease became the first neurodegenerative disease to be treated effectively by neurotransmitter replacement therapy.

However, there were drawbacks to the introduction of levodopa. Many patients had nausea and vomiting due to dopaminergic stimulation of the vomiting centres via the area postrema, which lies outside the blood-brain barrier. This difficulty was overcome in most patients by the introduction of selective extracerebral decarboxylase inhibitors, such as carbidopa in 'Sinemet', and benserazide in 'Madopar'. The next advance was the design of synthetic directly acting dopamine agonists, such as bromocriptine, lisuride, or pergolide, to stimulate cerebral dopamine receptors, followed by agents designed to enhance and prolong the duration of action of dopamine in the brain by inhibition of its major catabolic enzyme monoamine oxidase B—eg, selegiline hydrochloride (deprenyl).

ADDRESS: University Department of Clinical Neurology, The National Hospital for Nervous Diseases, Queen Square, London WC1N 3BG, UK (Prof C. D. Marsden, FRS).

The large range of drugs available was believed to be palliative, mostly by restoring striatal dopamine levels or activity; none was thought to protect against the basic cause of Parkinson's disease, which was unknown. Additionally, there were obvious difficulties with long-term levodopa therapy.[6,8,9] Many patients had unwanted side-effects of the drug and there were fluctuations in response. In the late 1970s and 1980s, many strategies were devised to try to prevent or overcome these long-term problems, particularly the early use of directly acting dopamine agonists, and restricting and delaying levodopa therapy to reduce the risk of getting dyskinesias and fluctuations. To smooth out and prevent fluctuations, longer acting forms of levodopa have been introduced ('Madopar CR' and 'Sinemet CR'), and patients with severe fluctuations can be stabilised by continuous administration of levodopa, PHNO [(+)-4-propyl-9-hydroxynaphthoxazine], lisuride, or apomorphine.[10-13] Overcoming these severe fluctuations has also been the stimulus to the development of brain grafting techniques to replace striatal dopaminergic delivery.[14,15]

In the 1980s there were two important new pieces of information which gave insight into the cause of Parkinson's disease. The first was the realisation that inheritance, in its simplest sense, was not a major factor in the aetiology of the disease. It had been assumed that genetic factors were important because at least 10–15% of cases had relatives similarly affected. However, monozygotic twins, when one had Parkinson's disease, were no more likely to share the illness than were dizygotic twins.[16] These data suggest that simple mendelian genetic factors do not have a major role, although more complex genetics may be involved and are a focus of increasing attention.

What about the environment? In 1983, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was identified as a human neurotoxin that could selectively destroy the substantia nigra, induce neuropathological and neurochemical changes very like those of Parkinson's disease, and cause a clinical illness similar to Parkinson's disease itself.[17] MPTP itself is not the active neurotoxin; it must be converted via MPDP by monoamine oxidase B, perhaps in glia, into 1-methyl-4-phenylpyridine (MPP^+).[18] MPDP is a substrate for the dopamine neuronal reuptake system, which normally inactivates dopamine released into the synaptic cleft at nigrostriatal terminals.[19] MPP^+ is thus concentrated into dopaminergic neurons where it binds to neuromelanin[20]—a byproduct of dopamine synthesis found in nigral nerve cells, particularly in primates and in greatest concentration in man. How does MPP^+ kill those nerve cells? Two complimentary mechanisms have been proposed. MPP^+ is taken up and concentrated in mitochondria, where it poisons NADH-linked components of complex I of mitochondrial energy metabolism,[21] leading to depletion of ATP and alterations in cellular calcium content. MPP^+ synthesis from MPTP also may induce the formation of free radical species, imposing oxidative stress with consequent lipid membrane peroxidation.[22] Perhaps MPP^+ poisons mitochondrial complex I activity as a result of free radical damage. Alternatively, dopamine catabolism by monoamine oxidase B generates hydrogen peroxide, and thus imposes oxidative stress on nigral neurons,[23] which are usually protected by various enzymes (eg, superoxide dismutase, catalase) and free radical scavengers (eg, reduced glutathione, vitamins E and C); with normal ageing these mechanisms may be insufficient, so that dopaminergic nigral neurons may have a tendency to self-destruct.

These observations have led to various hypotheses about the cause of Parkinson's disease; the simplest is that the illness is due to prolonged exposure to an MPTP-like neurotoxin in the environment. However, no such substance has yet been identified.[24] An alternative explanation is that there might have been toxic exposure early in life, even in utero, that caused subclinical nigral damage. The additive effects of normal nigral ageing might then cause a large enough additional nigral depletion to cause Parkinson's disease in later life.[25] Another theory is that the damaging agent may be a factor that can provoke extensive free radical formation in the substantia nigra. Excess iron, which has been found in the substantia nigra in the brains of people with Parkinson's disease,[26] may not be adequately bound in an inactive form, since the concentration of the major iron binding protein, ferritin, is reduced in the parkinsonian brain.

Attempts to slow the rate of disease progression has centred on treatment with deprenyl, with the aim to prevent free radical generation from dopamine metabolism and production of MPP^+-like compounds.[27]

Current understanding

Diagnosis of Parkinson's disease

The gold standard for Parkinson's disease is the pathological finding of a specific degeneration of nigral and other pigmented brainstem nuclei, with a characteristic inclusion, the Lewy body, in remaining nerve cells.[28] Most patients with a primary akinetic-rigid syndrome, usually with tremor, have this pathology of idiopathic Parkinson's disease at necropsy. However, about 10–15% of brains examined in the Parkinson's Disease Society Brain Bank have other conditions—namely, in descending order of frequency, multiple system atrophy (which encompasses the combined changes of strionigral, olivopontocerebellar, and intermediolateral column degeneration), Steele-Richardson-Olzewski disease, and coticobasal degeneration. The following features, though not specific, are the best predictors of Lewy body Parkinson's disease: a unilateral onset, classic rest tremor, and a pronounced response to levodopa therapy. An alternative diagnosis is based on the absence of rest tremor, no response to levodopa, early falls or dementia, prominent autonomic symptoms, and abnormalities of eye movement, cerebellar ataxia, or pyramidal signs. About 10–15% of groups of patients with Parkinson's disease diagnosed in life fail to respond to levodopa, and may have parkinsonism-plus syndromes, which are less responsive to treatment and have a poorer prognosis.

New epidemiological data

Previous estimates based on hospital referrals, which suggested that the incidence and prevalence of Parkinson's disease were similar in different communities, are unreliable. Apart from the issue of misdiagnosis, there may well be many undiagnosed patients in the community whose symptoms are dismissed as "old age". As many as 40% of cases may not reach hospital services. However, recent community-based surveys suggest that for unknown reasons the prevalence of Parkinson's disease may be lower in some countries than in others, notably China and Africa compared with Europe and North America.[24]

Although such epidemiological data are eagerly scanned in the search for environmental toxins, there are few

substantial leads. There are no obvious clusters of Parkinson's disease and the incidence of the disease has not changed much over the past 30 years, at least in middle America. A higher incidence of previous head injury and a lower prevalence of smoking has been found in some, but not all, surveys. However, these epidemiological methods may not be powerful enough to detect a multifactorial aetiology. The hypothesis that there may be an environmental insult many years before the clinical onset of the disease is not supported by observations on twins or spouses.[16] Since the prevalence of Parkinson's disease amongst monozygotic and dizygotic twins is similar to that in the rest of the population, exposure to an environmental agent in childhood or adolescence seems unlikely. Likewise, the occurrence of conjugal Parkinson's disease is remarkably low, even in marriages lasting many decades, so that environmental exposure in early or middle life seems improbable.

A more complex theory is that the development of Parkinson's disease may be due to a combination of exposure to an environmental toxin with an inherited inability to adequately dispose of such a toxin. Subtle differences in the activity of drug metabolising enzymes have been claimed in Parkinson's disease, and may be relevant.

Preclinical Parkinson's disease

The observation that Lewy bodies are also found in about 10% of brains from elderly controls who during life did not have the disease[29] could be taken as evidence against the specificity of the Lewy body for Parkinson's disease; the Lewy body could merely be an intraneuronal inclusion produced by a certain type of cell death, whatever the cause. By contrast, much indirect evidence has accumulated to support the notion that incidental Lewy bodies may be a marker of preclinical Parkinson's disease. Firstly, substantial loss of nigral neurons (50% or more) and striatal dopamine depletion (80% or more) in people dying with overt parkinsonism[24] point to a long period (perhaps 30 years or more) of nigral degeneration before the appearance of clinical symptoms. Secondly, in people with incidental Lewy bodies, there is histological evidence of nigral neuronal degeneration and cell loss in the same part of the substantia nigra affected as in those with clinically evident disease.[29] Finally, the incidence of incidental Lewy bodies increases with age parallel with that of clinical Parkinson's disease:[29] for every patient with overt Parkinson's disease, there are about 10 with incidental Lewy body disease.

This evidence, though not conclusive, suggests that there may be a large cohort of normal middle-aged and elderly individuals, who have preclinical Parkinson's disease, and who, if they live long enough, may get the disease in later life. The challenge, therefore, will be to develop simple methods to identify people with preclinical Parkinson's disease so that treatment could be given before the illness becomes evident.

New neuropathological and neurochemical clues to the cause of Parkinson's disease

Why are pigmented brainstem nuclei especially vulnerable? Neuromelanin is believed to be a derivative of catecholamine metabolism—eg, from dopamine in substantia nigra and noradrenaline in locus coeruleus—but degeneration in Parkinson's disease is not confined to catecholamine neurons. Thus, the cholinergic neurons in the substantia innominata are also affected regularly, and their loss may contribute to the cognitive changes and dementia seen in some patients. Likewise, histological studies with antibodies to ubiquitin (which helps to remove damaged proteins) indicate that the cortical changes are much more widespread than was formerly thought. Even within the substantia nigra compacta, which is the main focus of parkinsonian changes, neuronal cell loss is selective. The abnormalities in nigra begin in, and are greatest throughout, the ventrolateral region that projects to the putamen. The reasons why medial tegmental pigmented neurons, projecting to caudate, are relatively spared is uncertain.

The Lewy body may also provide clues: it contains phosphorylated neurofilaments, ubiquitin, phospholipids, and other cytoskeletal components. Lewy bodies differ in structure and content from other neuronal inclusions seen in neurodegenerative diseases such as neurofibrillary tangles of Alzheimer's disease or Pick bodies.

Histological studies indicate that cell destruction and degeneration continue throughout the course of the disease.[28,29] Neurochemical findings accord with these observations. Studies that have taken due care to control for postmortem artifacts have shown various relevant abnormalities in parkinsonian substantia nigra.

Oxidative stress generating free radicals finally damages cells by inducing peroxidation of lipids. There is evidence of increased and continuing lipid peroxidation in the parkinsonian nigra at the time of death.[30] This may be due to excessive production of free radicals, stimulated by excessive free iron,[26,31] or to inadequate defences against oxidative stress. Concentrations of vitamin E are normal in parkinsonian substantia nigra, but those of reduced glutathione have been claimed to be substantially lower (though, technical difficulties may have influenced these findings). Catalase activities are normal, whereas mitochondrial (but not cytosolic) superoxide dismutase activities are increased, which perhaps reflects an attempt to compensate for oxidative stress. Finally, a specific reduction of mitochondrial complex I activity has been found in substantia nigra of parkinsonian patients.[32] Such deficiency of complex I activity is similar to the suspected neurotoxic action of MPTP. That there was no change in the molecular mass of complex I polypeptides, points to a change in enzyme activity. Restriction fragment analysis of substantia nigra mitochondrial DNA has not shown any major deletion, but a small deletion or point mutation has not been excluded.

Further studies are needed to establish whether such changes are specific for Lewy body Parkinson's disease, whether they occur early in the illness (particularly in presymptomatic incidental Lewy body disease), and whether they may be due to drug therapy.

Management of Parkinson's disease

The newly diagnosed case

Until recently, all treatment of Parkinson's disease was palliative. Nothing was known to prevent or slow progression of the underlying pathology of the illness. However, a retrospective uncontrolled review of patients treated with the monoamine oxidase B inhibitor, deprenyl, in Europe had raised the possibility that this agent might slow progression by exerting a neuroprotective effect. In a very large controlled trial in 1989 deprenyl significantly

delayed the need for levodopa by about a year compared with placebo.[27] These results may be due to a symptomatic action of deprenyl, but they could indicate that deprenyl has a true neuroprotective effect by slowing the rate of progression of Parkinson's disease in newly diagnosed patients. Thus, for the first time, an agent has been found that may actually influence the underlying pathology of the disease. The implications of this discovery are immense. Firstly, all newly diagnosed cases of Parkinson's disease should be started on the drug to slow disease progression. Secondly, methods of diagnosing the illness at its earliest stage (or even before symptoms are present), should be developed. Any neuroprotective agent should be given as early as possible for maximum effect. Finally, improved neuroprotective agents need to be developed.

Which drugs should be given to patients with disabilities severe enough to need palliative treatment? Most patients will need such treatment within a few years. Anticholinergic drugs may be used to begin with, but are not nearly as effective as levodopa. In addition, there is the complication of mental side-effects, especially in the elderly patients, and the concern of giving cholinergic antagonists in a disorder with existing cortical cholinergic deficit. Amantadine has weak dopaminergic properties as well as its anticholinergic activity, and may have a temporary benefit. However, a dopamine agonist will eventually be needed in most cases. The choice is between levodopa (combined with a selective extracerebral decarboxylase inhibitor) and synthetic directly acting dopamine agonists. In general, levodopa is the most effective agent—nearly all patients with idiopathic Parkinson's disease will benefit. By contrast, only about a third of patients can be managed satisfactorily on, for example, bromocriptine alone. However, the major drawbacks to long-term levodopa therapy has led to a strategy of using low-dose levodopa with a dopamine agonist (eg, Sinemet or Madopar with bromocriptine[33] or lisuride[34]), which is now the preferred method of starting palliative treatment.

Complications of long-term therapy

After a few years of stable response to levodopa, the patient begins to experience end-of-dose deterioration or wearing-off a few hours after each dose of levodopa. In addition, various dyskinesias may have appeared, including early morning and off-period dystonias, diphasic chorea, ballism, dystonias or stereotypies, and peak dose movements.[8] Despite the usual measure to fractionate the dose of levodopa—smaller quantities more frequently—and also to add deprenyl and/or bromocriptine or newer longer acting preparations of levodopa, many patients begin to get increasingly severe and rapid oscillations in mobility and dyskinesias—the "on-off" effect. The afternoons are often the worst time, with prominent and disabling dyskinesias, and individual doses of levodopa failing to have any beneficial effect. Rescheduling protein intake or a low protein diet (to avoid competition of neutral amino acids with levodopa absorption and entry into the brain) are only partly and briefly effective in the control of these problems. Drug holidays, to restore dopamine receptor sensitivity, often lead to disastrous worsening of the Parkinson's disease during the withdrawal period.

Since the fluctuations in mobility and dyskinesias are related, at least partly, to peaks and troughs of plasma levodopa levels,[35] they can be overcome by continuous dopaminergic stimulation. Intravenous levodopa administration[10] is not a workable method and continuous intragastric or intraduodenal infusion is not generally suitable. Continuous subcutaneous infusion of the water-soluble D_2 dopamine agonist lisuride,[11] though successful in the control of motor fluctuations in many patients, produces unacceptable psychiatric side-effects. Continuous subcutaneous infusions of apomorphine, a combined D_1 and D_2 dopamine agonist, have been successfully used in parkinsonian patients with severe fluctuations.[12] However, many patients find the continuous battery-operated syringe-driver pump infusions too difficult to cope with. An alternative strategy is single subcutaneous injections of apomorphine (0·2–5 mg) with a 'Penject' system for patients in "off" periods. Oral domperidone may be given before these injections to prevent nausea and vomiting. Benefit usually appears within 5–15 min, and lasts 40–90 min. Patients are encouraged to take an adequate dose of a levodopa preparation every 3–4 h, and when they become immobile (turn off) to administer apomorphine by penject to produce mobility (switch on) until the next dose of levodopa works. This approach undoubtedly helps many advanced fluctuating parkinsonian patients to have a more stable day. Psychiatric problems have been uncommon, but the technique has not yet been perfected; dyskinesias frequently remain a major problem. Unfortunately, no drugs are yet available to prevent levodopa dyskinesias without antagonising the drug's benefit to mobility. Likewise, no selective antipsychotic medication has been found to control mental side-effects of antiparkinsonian drugs without increasing motor disability.

The future

Research into Parkinson's disease in the next decade will centre on improvements in neuroprotective treatment to prevent or slow the rate of progression of the disease; methods of protection against free radical damage; the role of excitatory amino acid antagonists, and specific methods of delivery of such agents to the brain; and early diagnosis for the most effective use of neuroprotective agents. Detection of preclinical Lewy body disease, for example with fluorodopa and positron emission tomography (PET) scanning,[36] may be possible; this technique can identify degrees of putaminal dopamine deficiency insufficient to produce overt clinical signs. PET technology is extremely expensive in its present form, but minicyclotrons dedicated to one or a few ligands, and cheaper PET cameras, are being developed. Other approaches to early diagnosis include the search for immunological markers of Lewy body degeneration in cerebrospinal fluid, the assessment of extracerebral dopamine metabolism as an index of what is happening in the brain, and the possible detection of Lewy bodies in extracerebral tissues.

Neurotransplantation or brain grafting has restored function of the damaged brain in laboratory animals.[37] Grafts of fetal substantia nigra into the striatum deprived of its dopaminergic innervation by MPTP or 6-hydroxydopamine can survive, synthesise, and release dopamine, reinnervate host tissue, respond to host brain activity, and restore some (but not all) lost motor functions. Such grafts have been successful in rat to rat, primate to primate, and human to rat (with immunosuppression) experiments.

The first attempts to use the brain grafting approach to treat Parkinson's disease used adrenal autografts.[14] The idea was to transplant the patient's own adrenal gland into their

damaged striatum as a source of dopamine; one adrenal gland was implanted into the head of the caudate nucleus on one side at open operation. However, initial claims of success have not been replicated. Over 300 such procedures have now been done and we now have a clearer picture.[37,38] Firstly, there is a risk in the operation itself: complications of both the abdominal surgery and the neurosurgical approach have been reported in 10–20% of cases. Secondly, there has only been slight or inconspicuous benefit: patients continue to require levodopa therapy, but there may be some increase in duration of action of each dose of the drug, and some improvement in off period disability; the consensus now is that these modest benefits do not justify the risks. Finally, evidence from PET scanning in life and postmortem studies indicates that such adrenal grafts do not survive. However, they seem to provoke some degree of regeneration of nigrostriatal nerve terminals in the damaged striatum. Tissues other than the adrenal implanted into the denervated striatum have had a similar effect in experimental studies. It appears that implantation of tissue into the damaged striatum can generate some form of nerve growth activity to promote regeneration, and the search is on to discover what these factors may be. Once identified, they could be used to treat Parkinson's disease.

The first attempts to use human fetal nigral grafts to treat Parkinson's disease have also been done. Stereotactic procedures are used to implant suspensions of fetal nigral tissue, so that surgical risks are reduced to a minimum. Although results of the preliminary trials of this approach are conflicting, there now is good evidence for survival of such grafts in some circumstances,[15] but to find the best technique needs to be resolved. The following issues remain controversial: the age span of the human fetal nigral tissue that provides a viable transplant; the size of the fetal nigral implant needed; the best position of implants within the striatum; the time necessary for functional development of the implants; the need for immunosuppression; and the ethics of using aborted human fetal tissue for grafting. Much further work is required to refine this procedure and its role in treatment is unlikely to be clear for many years.

REFERENCES

1. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967; 17: 427–42.
2. Bernheimer H, Birkmeyer W, Hornykiewicz O, et al. Brain dopamine and the syndromes of Parkinson and Huntington. *J Neurol Sci* 1973; 20: 415–45.
3. Hornykiewicz O. Dopamine and brain function. *Pharmacol Rev* 1966; 18: 925–64.
4. Agid Y, Javoy-Agid F, Ruberg M. Biochemistry of neurotransmitters in Parkinson's disease. In: Marsden CD, Fahn S, eds. Movement disorders 2. London: Butterworth, 1987: 166–230.
5. Cotzias GC, Van Woert MH, Schiffer LM. Aromatic amino acids and modification of parkinsonism. *N Engl J Med* 1967; 276: 374–78.
6. Marsden CD, Parkes JD. Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* 1977; i: 345–49.
7. Lees AJ. L-dopa treatment and Parkinson's disease. *Q J Med* 1986; 59: 535–47.
8. Marsden CD, Parkes JD. On-off effects in patients with Parkinson's disease on chronic levodopa therapy. *Lancet* 1976; i: 292–96.
9. Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The on-off phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. *N Engl J Med* 1984; 310: 483–88.
10. Quinn N, Parkes JD, Marsden CD. Control of on/off phenomenon by continuous intravenous infusion of levodopa. *Neurology* 1984; 34: 1131–36.
11. Obeso JA, Luquin MR, Martinez-Lage JM. Lisuride infusion pump: a device for the treatment of motor fluctuations in Parkinson's disease. *Lancet* 1986; i: 467–70.
12. Frankel JP, Lees AJ, Kempster PA, Stern GM. Subcutaneous apomorphine in the treatment of Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1990; 53: 96–101.
13. Coleman RJ, Lange KW, Quinn NP, et al. The antiparkinsonian actions and pharmacokinetics of transdermal (+)-4-propyl-9-hydroxy-napthoxazine (PHNO): preliminary results. *Movement Disorders* 1989; 4: 129–38.
14. Madrazo I, Drucker-Colin R, Diaz V, Martinez-Mata J, Torres C, Becerill JJ. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med* 1987; 316: 831–34.
15. Lindvall O, Brundin P, Widner H, et al. Grafts of foetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 1990; 247: 574–77.
16. Ward CD, Duvoisin RC, Ince SE, Nutt JD, Eldridge R, Calne DB. Parkinson's disease in 65 pairs of twins and in a set of quadruplets. *Neurology* 1983; 33: 815–24.
17. Ballard PA, Tetrud JW, Langston JW. Permanent human parkinsonism due to MPTP. *Neurology* 1985; 35: 949–56.
18. Chiba K, Trevor A, Castagnoli N. Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* 1984; 120: 574–78.
19. Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* 1985; 82: 2173–77.
20. D'Amato RJ, Benham DF, Snyder SH. Characterisation of the binding of N-methyl-4-phenylpyridine, the toxic metabolite of the parkinsonian neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to neuromelanin. *J Neurochem* 1987; 48: 653–58.
21. Vyas I, Heikkila RE, Nicklas WJ. Studies on the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Inhibition of NAD-linked substrate oxidation by its metabolite 1-methyl-4-phenylpyridinium. *J Neurochem* 1986; 46: 1501–07.
22. Rossetti ZL, Sotgiu A, Sharp D, Hadjiconstantinou M, Neff NH. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and free radicals in vitro. *Biochem Pharmacol* 1988; 37: 4573–74.
23. Cohen G, Spina MB. Deprenyl suppresses the oxidant stress associated with increased dopamine turnover. *Ann Neurol* 1989; 26: 689–90.
24. Tanner CM. The role of environmental toxins in the etiology of Parkinson's disease. *Trends Neurosci* 1989; 12: 49–54.
25. Calne DB, Langston JW. Aetiology of Parkinson's disease. *Lancet* 1983; ii: 1457–59.
26. Dexter DT, Wells FR, Lees AJ, et al. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J Neurochem* 1989; 52: 1830–36.
27. The Parkinson Study Group. Effect of deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 1989; 321: 1364–71.
28. Gibb WRG, Lees AJ. The significance of the Lewy body in the diagnosis of idiopathic Parkinson's disease. *Neuropathol Appl Neurobiol* 1989; 15: 27–44.
29. Gibb WRG, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988; 51: 745–52.
30. Dexter DT, Carter C, Wells FR, et al. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 1989; 52: 381–89.
31. Jenner P. Clues to the mechanism underlying dopamine cell death in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1989; 52 (special suppl): 22–28.
32. Schapira AHV, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1989; i: 1269.
33. Rinne UK. Early combination of bromocriptine and levodopa in the treatment of Parkinson's disease: a 5-year follow-up. *Neurology* 1987; 37: 826–28.
34. Rinne UK. Lisuride, a dopamine agonist in the treatment of early Parkinson's disease. *Neurology* 1989; 39: 336–39.
35. Nutt JG, Woodward WR. Levodopa pharmacokinetics and pharmacodynamics in fluctuating parkinsonian patients. *Neurology* 1986; 36: 739–44.
36. Brooks DJ, Frackowiak R. PET and movement disorders. *J Neurol Neurosurg Psychiatry* 1989; 52 (special suppl): 68–77.
37. Lindvall O. Transplantation into the human brain: present status and future possibilities. *J Neurol Neurosurg Psychiatry* 1989; 52 (special suppl): 39–54.
38. Goetz CG, Olanow CW, Koller WC, et al. Multicentre study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. *N Engl J Med* 1989; 320: 337–41.

EXHIBIT M

DRUG THERAPY

Drugs & Aging 1996 Sep; 9 (3): 169-184
1170-229X/96/0009-0169/$08.00/0

104

New Directions in the Drug Treatment of Parkinson's Disease

Jean-Louis Montastruc, Olivier Rascol and *Jean-Michel Senard*

Laboratory of Medical and Clinical Pharmacology, INSERM U317, Faculty of Medicine, University of Toulouse, and Clinical Pharmacology Service, University Hospital of Toulouse, Toulouse, France

Contents

Summary 169
1. Pathophysiology 170
2. Diagnosis 171
3. Emerging New Strategies and New Drugs 171
   3.1 Symptomatic Treatment 171
   3.2 Neuroprotective Strategies 178
   3.3 Prevention and Early Diagnosis 180
4. Conclusions 181

Summary

Parkinson's disease, a clinical syndrome with 4 cardinal features (bradykinesia, resting tremor, increased muscular rigidity and impaired postural balance), is mainly caused by the loss of dopaminergic neurons in the substantia nigra pars compacta. Although levodopa remains the 'gold standard' in the treatment of the disease, several emerging strategies are currently being developed. The first concerns new symptomatic drugs that either potentiate the effects of levodopa (e.g. slow-release preparations of levodopa, catechol-*O*-methyltransferase inhibitors and new dopamine agonists) or target clinical symptoms resistant to dopaminergic drugs (e.g. glutamate antagonists). The second strategy is to find drugs that are able to prevent or delay the neuronal death observed in Parkinson's disease. Several neuroprotective drugs are now in development in experimental research, but clinical trials in this area are still lacking. The development of these new drugs also depends on the validation of new clinical methodologies.

Parkinson's disease (PD), one of the commonest causes of disability among the elderly, is usually defined as a clinical syndrome with 4 cardinal features: bradykinesia (slowness and poverty of movement); resting tremor; rigidity; and abnormalities of posture and gait. Since the discovery of the beneficial effects of levodopa 35 years ago, few areas in pharmacology and medicine have surpassed PD in terms of progress in our understanding of the mechanisms involved and in drug treatment. Major advances in the pharmacological treatment of PD, and especially the introduction of levodopa, have markedly reduced morbidity and are an example of the triumph of rational pharmacology. This article reviews some recent aspects of PD, including advances in drug research and a discussion of future strategies for the treatment of this disease.

Table I. UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (from Gibb & Lees,[6] with permission)

Step 1. Diagnosis of parkinsonian syndrome
Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)
And at least 1 of the following:
muscular rigidity
4-6Hz resting tremor
postural instability not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction
Step 2. Exclusion criteria for Parkinson's disease
History of repeated strokes with stepwise progression of parkinsonian features
History of definite encephalitis
Oculogyric crises
Neuroleptic treatment at onset of symptoms
More than 1 affected relative
Sustained remission
Strictly unilateral features after 3 years
Supranuclear gaze palsy
Cerebellar signs
Early severe autonomic involvement
Early severe dementia with disturbances of memory, language and praxis
Babinski sign
Presence of cerebral tumour or communicating hydrocephalus on CT scan
Negative response to large doses of levodopa (if malabsorption excluded)
MPTP exposure
Step 3. Supportive prospective positive criteria for Parkinson's disease[a]
Unilateral onset
Resting tremor present
Progressive disorder
Persistent asymmetry affecting the side of onset most excellent response (70-100%) to levodopa
Severe levodopa-induced chorea
Levodopa response for 5 years or more
Clinical course of 10 years or more

a Three or more are required for a definite diagnosis of Parkinson's disease.

Abbreviations: CT = computerised tomography; MPTP = methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

1. Pathophysiology

The main hallmark of PD is the loss of dopaminergic neuron cell bodies in the zona compacta of the substantia nigra, leading to striatal dopamine denervation.[1,2] The dopaminergic defect explains the main clinical manifestations of PD (especially akinesia, rigidity and, to a lesser extent, tremor), although symptoms emerge only when such depletion exceeds 80 or 90%.

In addition to nigrostriatal dopamine denervation, postmortem studies have documented other biochemical abnormalities in the brains of patients with PD. These include mesocorticolimbic dopamine cell loss and decreases in hypothalamic dopamine levels. Changes in non-dopaminergic systems, such as reduced levels of several monoamines and peptides in the striatum, substantia nigra, globus pallidus, nucleus accumbens, limbic and/or cortical regions, hippocampus, cerebellar cortex and spinal cord, may also be responsible for some of the clinical characteristics of PD.[2,3] For example, the decrease in serotonin (5-hydroxytryptamine; 5-HT) and noradrenaline (norepinephrine) levels could explain the occurrence of depressive symptoms. It has also been proposed that the noradrenergic defect may explain the 'freezing' phenomena that occur during walking.

The cell loss observed in the intermediolateral cell column, the hypothalamus and the nucleus vagus dorsalis may be involved in the autonomic dysfunction observed in some patients with PD.[2,3] Peripheral autonomic changes, including hyposensitivity of α_2 adrenoceptors and hypersensitivity of peripheral vascular dopaminergic receptors with normal α_1 or β adrenoceptor sensitivity, have also been described.[4] However, the clinical consequences of all these central and peripheral changes remain unclear.

Recent knowledge about the interconnecting neuronal circuitries within the basal ganglia and the cellular compensatory effects of dopamine loss has focused interest on amino acids, and in particular glutamate, which is an excitatory neurotransmitter in the basal ganglia. A growing body of evidence suggests that striatal dopaminergic denervation results in overactivity of both the subthalamic nucleus and ventroanterior and ventrolateral nuclei of the thalamus, leading to reduced inhibitory input to the

Table II. Classification of parkinsonism (from Jankovic,[7] with permission)

Primary Parkinson's diseases
Idiopathic
tremor
postural instability and gait difficulty
akinesia ('freezing')
dementia
depression
sensory disturbance
autonomic dysfunction
Brain tumour
Trauma and pugilistic encephalopathy
Hydrocephalus (normal and high pressure)
Syringomesencephalia
Multiple system atrophies
Sporadic
progressive supranuclear palsy (ophthalmoparesis)
Shy-Drager syndrome (dysautonomia)
olivopontocerebellar atrophy (ataxia)
Parkinsonism-dementia-ALS complex (motor neuron disease)
striatonigral degeneration
corticodentatonigral degeneration with neuronal achromasia
Alzheimer's disease
Inherited
Huntington's disease
Wilson's disease
Hallervorden-Spatz disease
familial parkinsonism–dementia syndrome
familial basal ganglia calcification
neuroacanthocytosis
spinocerebellar-nigral degeneration and Joseph's disease
GDH deficiency

Abbreviations: ALS = amyotrophic lateral sclerosis; GDH = glutamate dehydrogenase.

cortex. Glutamate is believed to be the major neurotransmitter between the subthalamic nucleus and the substantia nigra, and between the substantia nigra and the thalamus.[5]

2. Diagnosis

Before considering recent advances in the pharmacology of PD, it is important to discuss the problems associated with its diagnosis. A review of the differential diagnosis of PD is beyond the scope of this article. However, in clinical practice, one of the first questions concerns the idiopathic nature of the disease and its response to levodopa. In fact, although the clinical features of PD are well known, there are at present no universally accepted pathognomonic diagnostic criteria. Indeed, as in Alzheimer's disease and other dementias, the diagnosis of idiopathic PD can be made only at post-mortem, with the detection of Lewy bodies. Brain-bank studies have identified some criteria that are helpful in the diagnosis of PD (table I).[6]

Symptoms of PD can also be associated with several other clinical conditions, for example dementia, depression, autonomic dysfunction and cerebellar disorders. Thus, classifications of parkinsonism usually differentiate between primary (idiopathic) PD, secondary parkinsonism and multiple system atrophy (MSA) [table II].[7,8] This review focuses only on recent advances in idiopathic PD.

3. Emerging New Strategies and New Drugs

The discovery of dopamine terminal loss in the striatum and the observation that the striatal cholinergic terminals remain fully active have led to the current pharmacological approach to treating PD. Treatment is aimed at restoring the disturbed neurotransmitter balance, either by: (i) inhibiting acetylcholine output with antimuscarinic drugs; or (ii) countering the dopamine deficiency through supplementation with levodopa and/or dopamine agonists. Recent pathophysiological studies have also allowed researchers to characterise some cellular mechanisms of neuronal death in the substantia nigra. Thus, interest in new therapeutic strategies includes research not only for new drugs but also for effective preventive strategies.

3.1 Symptomatic Treatment

In most patients with PD, the initial therapeutic success of levodopa is blunted by the development of motor and mental adverse effects after a few years of treatment.[4] Because of the limitations of levodopa therapy, the pharmacological treatment

of PD has been expanded to incorporate 4 different approaches:

- the development of new dopaminergic drugs, or the reassessment of old drugs in new strategies or delivery systems, to lessen the long-term adverse effects of levodopa;
- research for agents that target non-dopaminergic neurotransmitter systems;
- studies of the feasibility of brain transplants;
- new surgical approaches.

Only the first 2 areas are discussed in this article.

3.1.1 Enhancing Dopaminergic Transmission

Several clinical pharmacological studies have suggested that motor response complications are a consequence of both natural disease progression and the action of levodopa. It has been suggested that wearing-off responses appear to be primarily related to advancing degenerative changes affecting the dopaminergic nerve endings. Other fluctuations (e.g. peak-dose dyskinesias, or 'on-off' effects) could be explained by the chronic intermittent excitation (by levodopa treatment) of postsynaptic dopamine receptors, which are normally tonically stimulated.[9] Thus, strategies that improve dopaminergic neurotransmission could be useful to prevent or treat complications such as wearing-off responses.

Levodopa

Despite its long-term adverse effects, levodopa remains the 'gold standard' in PD patients. It is rapidly absorbed in the duodenum and transported across the gut wall by a saturable, facilitated carrier system (the aromatic and branched chain L-amino acid system).[10] Peak concentrations of the drug in plasma occur between 0.5 and 2 hours after an oral dose; there are, however, large inter- and intraindividual variations depending, for example, on the rate of gastric emptying and gastric pH.

Levodopa is always administered in combination with a peripherally acting dopa decarboxylase inhibitor (benserazide or carbidopa). This reduces some of the peripheral adverse effects of levodopa (cardiac arrhythmias, nausea, vomiting, etc.) and increases the fraction of administered levodopa that crosses the blood-brain barrier.

Because of rapid decarboxylation and the blood-brain barrier, less than 1% of the administered levodopa penetrates into the brain, even in the presence of a peripheral dopa decarboxylase inhibitor. Amino acids in the diet can compete with levodopa transport across both the intestinal mucosa and the blood-brain barrier. This observation led to the proposal to use low-protein diets in patients with levodopa-induced fluctuations in motor performance. Although the results of the studies are controversial, most of them show that this regimen increases the ratio of 'on' to 'off' hours.[11,12] Benefit usually occurs within a week of diet initiation.[11] The authors recommend that patients take levodopa with a carbohydrate-containing breakfast and lunch, and consume more protein with the evening meal.[12] This kind of regimen can be useful for some patients. However, since it often reduces protein intake, it could lead to malnutrition in elderly parkinsonian patients.

Another option is the use of slow-release preparations of levodopa (e.g. 'Sinemet CR', 'Madopar HBS'). 'Sinemet CR' is a mixture of levodopa 200mg and carbidopa 50mg in an erodible matrix that retards gastric tablet dissolution.[13] 'Madopar HBS' is a controlled-release tablet with levodopa 100mg and benserazide 25mg. When in contact with gastric fluid and after dissolution of the gelatin shell of the capsule, the 'hydrodynamically balanced system' (HBS) forms a mucous body with a bulk density of less than 1. This releases the drug at the desired rate while the dosage form remains in the stomach for a prolonged period of time.[14]

However, it is important to consider that 'Sinemet CR' and 'Madopar HBS' are not true extended-release dosage forms, but are formulations that produce attenuated peak plasma drug concentrations (C_{max}) and release the active compound slowly (i.e. they have reduced bioavailability). This indicates that the total daily dosage of levodopa may need to be increased by about 30% when switching from standard to slow-release levodopa.

Several studies have shown that 'Sinemet CR'[15,16] or 'Madopar HBS'[17,18] are effective drugs that prolong 'on' time, especially in patients with

wearing-off responses. Disadvantages of these slow-release preparations include delayed and poor responses after the first morning dose (it is often necessary to take a standard tablet as the first daily dose) as well as an exacerbation and prolongation of peak-dose dyskinesias (especially in the afternoon). The place of 'Sinemet CR' or 'Madopar HBS' as first-line treatment of PD in patients not previously treated with standard levodopa is not established.[19] Studies are now under way to investigate whether the more constant activation of dopaminergic neurons achieved by slow-release compared with standard levodopa could prevent or delay the occurrence of motor fluctuations.

Other researchers have sought to improve the delivery of levodopa to the gastrointestinal system or brain using intraduodenal, intravenous or intracerebroventricular infusion, or percutaneous administration.[20] Another exciting area of future research is the development of encapsulated dopamine-secreting cell lines, which could be implanted subcutaneously or into the striatum.[21]

Increasing Dopamine Synthesis

Clinical trials of dopamine precursors (e.g. tyrosine) or agents that stimulate tyrosine hydroxylase (tetrahydrobiopterin, oxyferriscorbone) have failed to give reproducible and significant results.

Enhancing Dopamine Release

Amphetamines are known to increase dopamine release from nigrostriatal terminals. However, these drugs, which have several adverse effects (hypertensive crisis, depression, dependence, etc.), have not been found to be useful in parkinsonian patients.[22]

Amantadine, first introduced as an antiviral agent for the prophylaxis of influenza, is a unique antiparkinsonian drug with multiple mechanisms of action: it enhances dopamine release and has antimuscarinic effects, but it may also increase dopamine synthesis and inhibit dopamine reuptake.[23] Recent studies also suggest that amantadine and the closely related compound memantine can block *N*-methyl-D-aspartate (NMDA) receptors.[24] Whatever its exact mechanism of action, the effects of amantadine on bradykinesia and tremor are modest and the drug is less effective than levodopa. Most patients fail to respond after several (3 to 6) months of treatment. However, amantadine is one of the only antiparkinsonian drugs possessing lateral psychostimulant properties, which can be useful in some patients.

Several authors have reported favourable results with electroconvulsive therapy (ECT) in patients with PD (for a bibliography, see Atre-Vaidya & Jampala[25]). ECT may improve motor as well as depressive symptoms.[25] However, most studies have so far been small and uncontrolled. Further study in this area is therefore needed.

Blocking Dopamine Reuptake

Blocking dopamine reuptake is known to increase dopamine levels in the synapse. However, drugs such as imipramine derivatives, amfebutamone (bupropion), mazindol and benzatropine (benztropine), which are known to block dopamine reuptake, have only a modest effect on motor symptoms in patients with PD.

Inhibiting Dopamine Metabolism

Most of a dose of orally administered levodopa is rapidly metabolised, first by peripheral dopa decarboxylase to dopamine (about 70%), which is further metabolised by intracellular monoamine oxidase (MAO) and extracellular catechol-*O*-methyltransferase (COMT).[10] The latter is present both peripherally and in the brain.

MAO is an enzyme that catalyses the oxidative deamination of various neurotransmitters such as catecholamines and serotonin. It exists in 2 forms. The B form, mainly located in platelets and the striatum, is responsible for the degradation of dopamine and other phenylethylamines, whereas the A form degrades serotonin. At low to moderate dosages (≤10 mg/day), selegiline (deprenyl) is a selective inhibitor of MAO-B and is free of the 'tyramine effect' common to other MAO inhibitors. It acts as an irreversible 'suicide' inhibitor of the enzyme. Its mechanisms of action have recently been reviewed.[26,27]

Selegiline has been used for many years as an adjunct to levodopa therapy, although its symptomatic benefit is relatively modest. It has been shown

to attenuate drug-induced motor fluctuations such as wearing-off effects in 50 to 70% of patients, and permits a 10 to 30% reduction in the total daily dosage of levodopa. In some patients, selegiline exacerbates peak-dose adverse effects of levodopa (e.g. dyskinesias, dystonia, confusion and hallucinations). Patients with severe, unpredictable on-off effects do not usually respond to selegiline. Used as monotherapy, this drug appears to be ineffective in some patients previously untreated with levodopa and effective in some other patients.[26] The putative neuroprotective effects of selegiline are discussed in section 3.3.

A recently published trial of selegiline has questioned the safety of the drug in PD.[28] A prospective study designed to compare the effectiveness of levodopa, bromocriptine and levodopa plus selegiline in early mild PD found a significantly higher mortality rate in the group of patients treated with selegiline plus levodopa compared with levodopa alone.[28] Until more information becomes available from this study, this surprising result must be interpreted cautiously, since several methodological biases could exist (number of lost patients, cause of death, risk α, etc.).[29]

Another potential source of bias in this study could be interactions between selegiline and antidepressants that inhibit serotonin reuptake. This would lead to so-called 'serotonin syndrome', characterised by anxiety, loss of consciousness, seizures or even pseudophaeochromocytoma crisis.[30] This interaction is well recognised with older serotonin reuptake inhibitors (e.g. fluoxetine) that are metabolised by cytochrome P450. Further studies are needed to investigate this interaction with newer serotonin reuptake inhibitors (e.g. citalopram).

Several other MAO inhibitors derived from amphetamine-like metabolites are currently in development as future antiparkinsonian drugs (e.g. lazabemide).[31] The development of mofegiline[32] has been stopped.

A series of new and selective COMT inhibitors has recently been developed. Entacapone, nitecapone and tolcapone are nitrocatechol-type agents active both *in vitro* and *in vivo*, whereas CGP 28014 is a pyridine derivative that is active only *in vivo*.[33] The main action of these agents is to inhibit the *O*-methylation of levodopa, thus improving its bioavailability and brain penetration, and potentiating its motor effects.[34] Entacapone and nitecapone have mainly peripheral effects, whereas tolcapone and CGP 28014 are also active in the brain.

Several recent clinical studies have shown that COMT inhibitors reduce plasma levels of 3-*O*-methyldopa, which is believed to compete with levodopa transport through the intestinal mucosa as well as through the blood-brain barrier. COMT inhibitors do not affect levodopa absorption (i.e. the maximum plasma concentration is unchanged), but they do prolong its half-life. This increases the duration of action of a single dose of levodopa, and for this reason COMT inhibitors may be useful as adjunctive therapy in PD.[35,36] COMT inhibitors may also facilitate a reduction in the dosage of levodopa.

Future studies must investigate the long-term beneficial and adverse effects of COMT inhibitors. For example, COMT inhibitors may worsen dyskinesias. In that case, the dosage of levodopa should be reduced or the dosage interval prolonged.

Continuous Dopamine Therapy

Two factors have led to the proposal that continuous treatment of PD could delay the onset of treatment complications. The first of these is the hypothesis that peak-dose dyskinesias and on-off effects can be explained by intermittent stimulation of the postsynaptic dopamine receptors.[9,37] Secondly, it has been observed that more constant activation of dopamine receptors, through continuous infusion of levodopa or dopamine agonists (which causes fewer receptor alterations than pulsatile dopaminergic stimulation), counteracts some motor fluctuations in patients with advanced PD.[36] Continuous dopaminergic stimulation could be achieved by a long-term infusion of levodopa or apomorphine (a dopamine agonist) in previously untreated PD patients. Of course, for evident reasons, such a possibility cannot be investigated in humans.

The goal of continuous dopamine stimulation could justify, from a theoretical point of view, the use of slow-release levodopa formulations as first-line treatment of the disease. As indicated above, the place of these formulations in previously untreated patients remains unknown, and the results of a large, multicentre, controlled trial comparing standard and slow-release levodopa in this patient group are awaited with interest. Another possibility could be long-acting dopaminomimetic drugs (dopamine agonists), which may offer a more physiological pattern of stimulation.

Dopamine Agonists

Because the efficacy of levodopa wanes over time in many PD patients, interest has turned to the development of drugs acting directly on dopamine receptors. Dopamine agonists include ergot derivatives, apomorphine and more recently developed drugs.

Ergot Derivatives

The current status of dopamine agonists in PD was recently reviewed.[38] Dopamine agonists are a heterogeneous group of drugs that produce their antiparkinsonian effect through the activation of dopamine receptors. They were first developed in the early 1970s and, to date, bromocriptine, lisuride and pergolide have undergone extensive clinical trials and are now used in clinical practice. These drugs are ergot derivatives. Dopamine agonists act directly on postsynaptic dopamine D_1 and/or D_2 receptors. The primary target of dopamine agonists is believed to be the D_2 receptor, although some recent studies have suggested a role for D_1 receptors in the treatment of PD.[39]

The binding profiles of dopamine agonists at D_1 and D_2 receptors differ slightly. Bromocriptine stimulates only D_2 receptors and is a partial agonist at D_1 receptors. Lisuride is a potent D_2 and a weak D_1 receptor agonist. Pergolide stimulates D_2 more than D_1 receptors.[38] Despite their different pharmacodynamic and pharmacokinetic profiles, the 3 drugs appear to be very similar in terms of their clinical efficacy.

This pharmacological profile suggests certain theoretical advantages of these drugs over levodopa: (i) they stimulate postsynaptic dopamine receptors directly, thus bypassing the degenerating nigrostriatal neurons; (ii) they do not depend on a pool of decarboxylase enzyme for conversion into the active transmitter; (iii) in contrast to levodopa, which yields 6-hydroxydopamine, they do not produce toxic metabolites; and (iv) their use will not result in the formation of free radicals, which have potential adverse consequences on disease progression. Moreover, their use permits reduction of the levodopa dosage.[38]

Dopamine agonists also have some clear disadvantages over levodopa: (i) they are less effective in reducing parkinsonian symptoms; (ii) they produce adverse effects (orthostatic hypotension, vasoconstriction, limb oedema, retroperitoneal fibrosis, psychiatric disorders); and (iii) they are more expensive.[38]

Dopamine agonists are usually used in combination with levodopa when late adverse effects occur, especially wearing-off effects, or when the efficacy of levodopa wanes.[38] They can also be prescribed as monotherapy in some patients, and relatively high dosages can be used as initial treatment. However, their efficacy often decreases after 1 to 3 years.[40] Another possibility is combination therapy, which is known to delay the onset of levodopa-induced late adverse effects (abnormal movements, fluctuations in daily motor performance).[41,42] At present, the best therapeutic strategy (i.e. early[1] *vs* late[2] combination with levodopa), and the place of these drugs compared with others such as selegiline, remains unknown.

Apomorphine

The rediscovery of apomorphine for the management of patients with PD is one of the best examples of the value of 'old drugs' in modern pharmacology. Apomorphine is the most potent dopamine agonist now available, acting at both D_1

1 Early combination treatment refers to initial treatment with a dopamine agonist plus levodopa.

2 Late combination treatment means initiating therapy with a dopamine agonist and adding in levodopa later on, reverting to dopamine agonist monotherapy when the efficacy of dopamine replacement declines.

and D_2 receptors.[43] Its long-term oral use has been associated with nausea, vomiting and azotaemia, and is limited by hepatic first-pass metabolism. In combination with domperidone, the subcutaneous administration of apomorphine produces a potent and rapid antiparkinsonian action. Apomorphine alleviates bradykinesia and rigidity as well as parkinsonian tremor.

Repeated subcutaneous injections of apomorphine (using a 'Penject' system) significantly reduce daily 'off' periods in patients with fluctuating motor function. Benefits occur within 5 to 15 minutes and last for 45 to 90 minutes. Apomorphine has relatively few adverse effects, and adverse psychiatric reactions seem to be less frequent than with other dopamine agonists. Markedly disabled patients with frequent fluctuations (>10 per day) can be managed with continuous daytime subcutaneous infusions of apomorphine via a syringe driver.[43]

Apomorphine can also be used as a test for dopaminergic responsiveness. This test has a good predictive value for subsequent responsiveness to levodopa treatment, and may help in the differential diagnosis of idiopathic PD and parkinsonian syndromes.[43]

Current research is ongoing into alternative routes of administration for apomorphine. It is effective by the sublingual route, but the time to its onset of action is longer than after subcutaneous injection.[43] Apomorphine has also been found to be effective after rectal or intranasal administration for treating 'off' periods.[43] Although the long-term adverse effects of apomorphine are unknown, these routes could be useful in patients unable to administer apomorphine by subcutaneous injection.[43]

New Dopamine Agonists

Several other dopamine agonists are currently under development,[44,45] including cabergoline [an ergoline derivative with a long half-life (24 hours)], and the non–ergot derivative ropinirole, which is a potent D_2 receptor agonist.[46] Pramipexole is a D_2 autoreceptor agonist that is under clinical development. In addition, the possible usefulness of dopamine partial agonists, such as terguride, is currently under investigation. Other selective D_1 and D_2 receptor agonists and transdermal preparations are also being studied.[44,45]

The development of dopamine agonists was a major step forward in the treatment of PD, but the ideal drug (i.e. orally active, long-acting and as potent as levodopa) has not yet arrived. Such an ideal drug should induce fewer motor complications than levodopa, have no psychiatric adverse effects, and could be used alone to specifically stimulate central dopamine receptors.[38]

It will be necessary to reassess the pharmacological profile of existing and future dopamine agonists in accordance with the new molecular classification of the 5 known dopamine receptor subtypes.[47] At present, the roles of the different receptor subtypes remains unknown, although in primates the D_3 receptor is present in significant numbers in the caudate-putamen. Both D_1 and D_2 receptor agonists induce dyskinesias in drug-naive or levodopa-treated animals. Recent experimental studies suggest that low dosages of D_1 receptor agonists might have antiparkinsonian effects without inducing dyskinesias. On repeated administration, such treatment diminished the intensity of dyskinesias in levodopa-primed, methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated primates.[39,48]

In previously untreated patients with PD, D_1 receptor agonists exert a mild antiparkinsonian effect, mainly reducing tremor.[49] It has been suggested that a strategy which includes D_1 receptor activation and reduces the sensitivity of the D_2 receptor could provide good symptomatic control and reduce dyskinesias in patients with advanced PD.[37] Clinical studies are needed to confirm this, however.

3.1.2 Manipulating Non-Dopaminergic Neurotransmitters

As discussed in section 1, several significant biochemical changes in non-dopaminergic systems have been described in patients with PD. Thus, many workers have tried to manipulate neurotransmitters other than dopamine and acetylcholine

to find evidence for an adjuvant effect of levodopa or effects on parkinsonian symptoms that do not respond well to levodopa [e.g. gait disorders (freezing), postural instability, dysarthria, cognitive disorders].

Cognitive disorders in parkinsonian patients probably result from multiple alterations in neurotransmitter systems, involving for example noradrenaline, acetylcholine or even peptide transmitters. The manipulation of these neurotransmitters has not resulted in any observable improvement of cognition in patients with PD. In contrast, the serotonin precursor 5-hydroxytryptophan (5-HTP) and serotonin reuptake inhibitors (e.g. fluoxetine) have been shown to provide a mild antidepressant effect in patients with PD.[50] The favourable effect of progabide, a γ-aminobutyric acid agonist, suggested by Bartholini et al.[51] has not been confirmed by others.[51] Naltrexone, an opiate antagonist, was found to be ineffective in PD in patients with and without dyskinesias.[52]

More interesting is the discussion of the clinical correlation between central noradrenergic depletion and PD. This could explain the occurrence of depression, dementia, motor blocks (freezing) and orthostatic hypotension in patients with PD. For example, Japanese authors have reported that droxidopa (dihydroxyphenylserine; L-threo-dops) can improve freezing phenomena,[53] although this beneficial effect was not confirmed by others. Since droxidopa is an immediate precursor of noradrenaline, it may improve the orthostatic hypotension often observed in patients with PD.[54]

It was recently suggested that noradrenaline may play a neuroprotective role in PD,[55] since lesions of the locus coeruleus by 6-hydroxydopamine in MPTP-treated monkeys are associated with a more marked dopamine depletion and greater substantia nigra cell loss compared with nonlesioned controls.[56] The locus coeruleus may have a protective effect on nigral dopaminergic neurons. Since it is known that α_2 adrenoceptors inhibit the release of dopamine from the caudate nucleus,[57] it could be suggested that α_2 adrenoceptor antagonists could exert beneficial (neuroprotective and/or symptomatic) effects in PD. Preliminary studies are encouraging[58] but unequivocal evidence is still awaited.

Recent physiological studies have demonstrated the importance of excitatory amino acids, particularly glutamate, in the basal ganglia. For example, the excessive output from the subthalamic nucleus to the internal pallidal segment, and to the reticular part of the substantia nigra, that occurs in PD is mediated by glutamate.[5] Animal studies have shown that NMDA receptor antagonists potentiate the effects of levodopa and can protect the substantia nigra from MPTP-induced neurotoxicity in rats. Injection of the NMDA receptor antagonist dizocilpine (MK 801) within the medial pallidum reverses parkinsonian symptoms in MPTP-treated monkeys.[59]

The mechanism of action remains unclear, but it has been suggested that blockade of NMDA receptors facilitates dopamine action by preventing the glutamate-induced dephosphorylation of DARPP-32, a dopamine- and cyclic adenosine monophosphate (cAMP)–regulated phosphoprotein.[59] Despite some experimental evidence, clinical data are still lacking because the few clinical studies that have been conducted with NMDA receptor antagonists have failed to demonstrate any favourable effect in patients with PD.[60,61] Further studies with new drugs are needed.

A frequently observed long-term complication of treatment with levodopa in patients with advanced PD is the occurrence of mental disturbances – hallucinations, vivid dreams, paranoid ideation and delirium. These psychiatric symptoms reflect the consequences of dopaminergic stimulation on an underlying degenerative neuropathological process. They can be improved by reducing the levodopa (and/or dopamine agonist) dosage or by adding antipsychotics, although these invariably worsen the parkinsonian symptoms.

Clozapine is an atypical antipsychotic developed more than 30 years ago, which was withdrawn from clinical use because of its ability to cause bone marrow suppression. It is now under reinvestigation in the management of psychosis

refractory to standard treatment. In contrast to other antipsychotics, clozapine acts as a relatively selective D_1 antagonist. It also blocks the D_4 receptor in the limbic system and possesses potent antimuscarinic activity, which may explain the low incidence of extrapyramidal adverse effects associated with its use.

Consequently, several authors have investigated the use of clozapine in patients with PD and psychotic symptoms.[62,63] The results were reported to be favourable, although the studies were performed with a large range of dosages (6.5 to 250 mg/daily) on a relatively small number of patients without double-blinding.[62,63] Despite several adverse effects [hypotension, tachycardia, sialorrhoea and, more importantly, granulocytopenia (which occurs in 1 to 5% of patients during treatment)], clozapine represents an important therapeutic advance in some PD patients with mental disorders after long-term levodopa treatment.

3.2 Neuroprotective Strategies

The precise mechanism of neuronal death in PD remains unknown. Several factors are involved, including oxidative stress, mitochondrial abnormalities, calcium cytotoxicity, iron accumulation, excitotoxic or immunological factors.[64,65] These biochemical observations have resulted in new therapeutic strategies aimed at preventing the natural progression of the disease.[65]

3.2.1 Antioxidative Properties of Selegiline

The discovery that selegiline can prevent the neurotoxic effects of MPTP in animals led to the reassessment of selegiline in previously untreated patients with PD. It was speculated that selegiline might alter the progression of the disease by reducing the generation of potentially neurotoxic substances from either endogenous or exogenous compounds.

Birkmayer et al.[66] found that patients treated with selegiline plus levodopa survived 12% longer than those treated with levodopa alone. However, this was a retrospective, uncontrolled study that had major methodological defects. Tetrud and Langston[67] reported the results of a double-blind, placebo-controlled trial of selegiline in 54 patients with early PD who had never received levodopa. Patients treated with selegiline for 3 years were able to do without levodopa for 549 days, compared with 312 days for those receiving placebo. The rates of progression of 4 clinical rating scales were slowed by between 40 and 64%.

The most interesting results come from the DATATOP study.[68] This study compared the effects of placebo and selegiline on the progression of disability in early PD. An interim analysis revealed that the rate of reaching the end-point (i.e. adding levodopa) was much slower in the selegiline-treated group than in the placebo group. The final report showed that the beneficial effects of selegiline, which occurred largely during the first 12 months of treatment, remained strong and significantly delayed the onset of disability requiring levodopa therapy. The between-group difference in the estimated median time to the end-point was about 9 months. The ratings for PD improved during the first 3 months of selegiline treatment, and the motor performance of selegiline-treated patients worsened after treatment was withdrawn.[68] The authors concluded that selegiline 10 mg/day delays the onset of disability associated with early, otherwise untreated, PD.

This study raises a number of methodological, clinical and pharmacological questions. From a methodological point of view, the criteria selected (probability of reaching the end-point and ceasing full-time employment) have not been validated by previous studies. The clinical significance of the study also remains debatable because the long-term (5 to 10 years) consequences remain unknown. Finally, the mechanism of action (antidepressant, symptomatic, protective or all of these) of selegiline is unclear[69] (table III).

Two clinical trials have recently reinvestigated the neuroprotective effects of selegiline in PD.[70,71] The SINDEPAR trial[70] (performed on 86 previously untreated patients) is comparing 4 treatments in PD patients: (i) selegiline plus levodopa; (ii) selegiline plus bromocriptine; (iii) placebo plus levodopa; and (iv) placebo plus bromocriptine.

Table III. Possible mechanisms of action of selegiline (from Jankovic,[69] with permission)

Enhancement of dopaminergic transmission
Inhibits oxidation of MPTP to MPP^+
Produces amphetamine-like effect
enhances release of dopamine
blocks reuptake of dopamine
Increases striatal phenylethylamine levels
enhances release of dopamine
activates dopamine receptors
Stimulates gene expression of L-amino acid decarboxylase in PC12 cells
Neuronal protection
Reduces production of oxidative radicals
Up-regulates superoxide dismutase and catalase
Suppresses nonenzymatic, iron-catalysed auto-oxidation of dopamine and polymerisation of dopamine-melanin
Neuronal rescue
Compensates for loss of target-derived trophic support (stereospecific)
Enhances glial activation
Induces NT-3trikC receptor
Up-regulates CNF gene expression in astroglial cell culture
Delays apoptosis in serum-deprived PC12 cells
Blocks apoptosis-related fall in mitochondrial membrane potential

Abbreviations: CNF = ciliary neurotrophic factor; MPP^+ = active metabolite of MPTP; MPTP = methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

This study included a 7-week wash-out period of selegiline to exclude a symptomatic effect. Motor deterioration was more pronounced in the 2 placebo groups, suggesting that selegiline can delay the progression of signs and symptoms via a mechanism that is not readily accounted for by its symptomatic effects.[70]

The second trial[71] is a long-term, controlled study being performed in Finland, comparing the need for levodopa in previously untreated patients given placebo or selegiline for 4 years. It showed that the levodopa dosage was 58% higher in the placebo group than in the selegiline group, with a lower daily levodopa intake and end-of-dose deterioration in the latter.[71]

This study needs to be completed, and further trials are required to better define the respective roles of selegiline and other drugs (e.g. dopamine agonists) that can be used as first-line treatment in the early management of PD.

Other studies have failed to find evidence for a neuroprotective effect of selegiline. For example, Brannan and Yahr[72] compared 2 groups of PD patients, the first treated with levodopa alone and the second treated initially with selegiline and then subsequently started on levodopa therapy on an as-needed basis. For a similar symptomatic effect, the sole difference after 3 to 5 years of treatment was that patients in the selegiline plus levodopa group received less levodopa than patients who received monotherapy.[72]

The DATATOP study[68] also investigated the effect of tocopherol (vitamin E), which traps free radicals, in patients with PD. The study failed to show any beneficial effect of tocopherol or any interaction between selegiline and tocopherol.[68]

3.2.2 Antioxidative Properties of Dopamine Agonists

Recent literature also discusses the putative neuroprotective actions of dopamine agonists.[39] Pergolide, like selegiline, elevates superoxide dismutase activity in the brain, decreases hydrogen peroxide formation from dopamine and preserves nigral cells in aging rats.[73-75] Bromocriptine, apomorphine and other agonists also scavenge free radicals and have antioxidant activity, in contrast with the mainly pro-oxidant actions of levodopa.[76] However, these studies are only *in vitro* experiments, and the clinical consequences of such properties remain to be determined.

3.2.3 Experimental Interventions Affecting Brain Iron

The demonstration that iron, which accumulates in the substantia nigra in patients with PD, can exert direct toxic effects on nigral neurons led to the search for new pharmacological approaches. These approaches are: (i) reducing the entry of iron into the brain; (ii) increasing the nontoxic storage of iron; (iii) removing (chelating) accumulated iron; and (iv) a combination of these interventions.[65] No clinical data using such therapeutic strategies have yet been published.

Table IV. Potential neuroprotective therapies for Parkinson's disease (from Jankovic,[69] with permission)

Strategy	Example
Levodopa-sparing strategies	Dopamine agonists
Dopamine receptor agonists	Bromocriptine Lisuride Pergolide
Dopamine transport inhibitors	Mazindol 21-Aminosteroids
Antioxidants	MAO-A and MAO-B inhibitors
Lipid peroxidation inhibitors (free radical scavengers)	Tocopherol (vitamin E) Ascorbic acid (vitamin C) β-Carotene Lazaroids 21-Aminosteroids
Free radical trappers	Phenylbutylnitrone
Calcium antagonists	Dihydropyridines
Iron chelators	Deferoxamine (desferrioxamine) 21-Aminosteroids
Glutamate antagonists	NBQX Remacemide Amantadine Certain anticholinergic drugs Lamotrigine
Trophic factors	BDNF IGF FGF EGF GM1 gangliosides GDNF
Restorative therapy with brain implants	
Subthalamotomy	
Control of potential risk factors	

Abbreviations: BDNF = brain-derived neurotrophic factor; EGF = epidermal growth factor; FGF = fibroblast growth factor; GDNF = glial cell line–derived neurotrophic factor; IGF = insulin-like growth factor; MAO = monoamine oxidase; NBQX = 2,3-dihydroxy-6-nitro-7-sulfamoyl benzo(f)quinoxaline.

3.2.4 Other Strategies

Several other mechanisms have been reported to be involved in nigral cell death in patients with PD. These include a possible role for MPTP-like bioactivated neurotoxins and abnormalities in the enzymes regulating the metabolism of xenobiotic substances, particularly the hepatic cytochrome P450 system.[77,78] These observations led several investigators to propose that the evolution of the disease could be prevented via various methods, which were recently reviewed in *CNS Drugs*:[78] MAO inhibitors, dopamine transporter blockers, glutamate receptor antagonists, calcium antagonists, glutathione-type drugs and neurotrophic factors[78] (table IV).

Growth factors (e.g. basic fibroblast growth factor) have been shown to have potent effects on grafted dopamine neurons in rats with experimental PD.[79] This kind of study also suggests new approaches for enhancing the survival and function of dopamine neuron grafts. However, no clinical data are yet available.

Moreover, as discussed in section 3.2.1, new clinical methodologies for investigating a new drug with putative neuroprotective properties need to be developed.

3.3 Prevention and Early Diagnosis

The most important medical challenge in PD is to prevent the occurrence of the disease. The first area of this research is the study of drugs and toxins known to cause parkinsonism. In fact, of the secondary parkinsonisms, the most frequently observed clinical situation is drug-induced parkinsonism. It is often difficult to clinically differentiate idiopathic and drug-induced parkinsonism. Recent data indicate that, besides classical antipsychotics, parkinsonism can also be caused by calcium antagonists and some psychotropic and cardiotropic drugs.[80]

The drugs listed in table V must be avoided in patients with PD. Investigations into the mechanism of drug-induced parkinsonism could lead to new discoveries in the pathophysiology of the disease. For example, it has been shown that haloperidol and chlorpromazine inhibit complex I *in vitro* in rat brain mitochondria as well as in platelets.[80]

It would also be advantageous to be able to detect idiopathic PD before symptoms develop. Several techniques, such as radioimaging (positron emission tomography or single photon emission tomography), physiological and biochemical tests, are currently being studied but no definite conclusions can be reached at present.[81,82]

Table V. Summary of the main data concerning drug-induced parkinsonism (from Montastruc et al.,[80] with permission)

Pharmacological class	Drugs	Intensity of the parkinsonian syndrome	Mechanism of action	Comments
Antiadrenergic drugs	Reserpine	+++	Deplete dopamine stores	
	Tetrabenazine	+++	Deplete dopamine stores	
Antipsychotics	Phenothiazines	++	Dopamine receptor blockade	
	Butyrophenones	+++	Dopamine receptor blockade	
	Thioxanthenes	++	Dopamine receptor blockade	
	Dibutylpiperidines	+++	Dopamine receptor blockade	
	Benzamides	+	Dopamine receptor blockade	Very rare with metopimazine and domperidone
	Clozapine	0 to +	Dopamine receptor blockade/antimuscarinic properties	
	Loxapine	++	Dopamine receptor blockade	
Antihypertensives	Methyldopa	0 to +	False neurotransmission	Rare
Calcium antagonists	Flunarizine	0 to +	Dopamine receptor blockade	Very rare with diltiazem or verapamil; dihydropyridines are also unlikely to cause this effect
	Cinnarizine	0 to +	Dopamine receptor blockade	
Antidepressants	Fluoxetine	0 to +	?	Not with imipramine derivatives
Antiarrhythmics	Amiodarone	0 to +	?	Yet to be confirmed

Symbols: 0 indicates absent; + indicates slight; ++ indicates marked; +++ indicates very marked; ? indicates that the mechanism of drug-induced parkinsonism remains to be determined.

4. Conclusions

New strategies for improving the treatment of PD are currently under development. These include drugs acting on the classical pharmacological target in PD (the nigrostriatal dopamine system): controlled-release preparations of levodopa, COMT inhibitors and new dopamine agonists, as well as new routes of administration of established dopaminergic drugs. A second goal is to find drugs that improve or correct symptoms resistant to levodopa therapy, such as freezing, falls and orthostatic hypotension. Thirdly, the major challenge remains to find a true neuroprotective drug in order to retard or prevent free radical damage.

Finally, the management of PD must aim to maintain an equilibrium between the satisfactory control of extrapyramidal symptoms and the long-term effects of levodopa administration. The 2 most exciting challenges in the future pharmacological approach to PD may lead to: (i) the development of effective antiparkinsonian drugs that do not exhibit the long-term adverse effects associated with levodopa (abnormal movements, fluctuations in performance, psychosis); and (ii) the introduction of true 'antidyskinetic' agents that do not aggravate extrapyramidal symptoms, as is the case with currently available antipsychotics.

These points underline the need for new, effective drugs and new clinical methodologies for drug evaluation, in addition to well-performed, long-term pharmacoepidemiological trials to test the numerous hypotheses raised by the growing field of basic neuroscience.

Acknowledgements

The authors would like to thank Mrs P. Bontemps for her meticulous preparation of the manuscript.

References

1. Agid Y, Javoy-Agid F, Ruberg M. Biochemistry of neurotransmitters in Parkinson's disease. In: Marsden CD, Fahn S, editors. Movement disorders. Vol. 2. New York: Raven Press, 1987: 166-230
2. Hirsch EC. Biochemistry of Parkinson's disease with special reference to the dopaminergic systems. Mol Neurobiol 1994; 9: 135-42
3. Agid Y, Cervera P, Hirsch E. Biochemistry of Parkinson's disease 28 years later: a critical review. Mov Disord 1989; 4 Suppl. 1: S126-44
4. Montastruc JL. Recent advances in the clinical pharmacology of Parkinson's disease. Therapie 1991; 46: 293-303
5. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989; 12: 366-75
6. Gibb WRG, Lees A. The relevance of Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988; 51: 745-52
7. Jankovic J. The relationship between Parkinson's disease and other movement disorders. In: Calne DB, editor. Drugs for the treatment of Parkinson's disease. Hand Exp Pharmacol 1988; 88: 227-70
8. Quinn N. Multiple system atrophy: the nature of the beast. J Neurol Neurosurg Psychiatry 1989; Suppl.: 78-89
9. Chase TN, Baronti F, Fabbrini G, et al. Rationale for continuous dopaminomimetic therapy of Parkinson's disease. Neurology 1989; 39 Suppl. 2: 7-10
10. Nutt JG, Fellman JH. Pharmacokinetics of levodopa. Clin Neuropharmacol 1984; 7: 35-49
11. Riley D, Lang AE. Practical application of a low protein diet for Parkinson's disease. Neurology 1988; 38: 1026-31
12. Kurlan R. Dietary therapy for motor fluctuations in Parkinson's disease. Arch Neurol 1987; 44: 1119-21
13. Dempski RE, Scholtz EC, Oberholtzer ER, et al. Pharmaceutical design and development of a Sinemet controlled-release formulation. Neurology 1989; 39 Suppl. 2: 20-4
14. Erni W, Held K. The hydrodynamically balanced system: a novel principle of controlled drug release. Eur Neurol 1987; 27 Suppl. 1: 21-7
15. Deleu D, Jacques M. Controlled-release carbidopa/levodopa (CR) in parkinsonian patients with response fluctuations on standard levodopa treatment: clinical and pharmacokinetic observations. Neurology 1989; 39 Suppl. 2: 88-91
16. Feldman RG, Mosbach PA, Kelly MR, et al. Double blind comparison of standard Sinemet and Sinemet CR in patients with mild-to-moderate Parkinson's disease. Neurology 1989; 39 Suppl. 2: 96-100
17. Marion MH, Stocchi F, Malcolm SL, et al. Single-dose studies of a slow-release preparation of levodopa and benserazide (Madopar HBS) in Parkinson's disease. Eur Neurol 1987; 27 Suppl. 1: 28-35
18. Rondot P, Ziegler M, Aymard N, et al. Clinical trial of Madopar HBS in parkinsonian patients with fluctuating drug response after long-term levodopa therapy. Eur Neurol 1987; 27 Suppl. 1: 114-9
19. Rinne UK, Rinne JO. Treatment of early Parkinson's disease with controlled-release levodopa preparations. Neurology 1989; 39 Suppl. 2: 78-81
20. Sage JI, Trooskin S, Sonsalla PK, et al. Experience with continuous enteral levodopa infusions in the treatment of 9 patients with advanced Parkinson's disease. Neurology 1989; 39 Suppl. 2: 60-2
21. Sabel BA, Dominiak P, Hauser W. Levodopa delivery from controlled-release polymer matrix: delivery of more than 600 days *in vitro* and 225 days of elevated plasma levels after subcutaneous implantation in rats. J Pharmacol Exp Ther 1990; 255: 914-22
22. Parkes JD, Tarsy D, Marsden CD. Amphetamines in the treatment of Parkinson's disease. J Neurol Neurosurg Psychiatry 1975; 38: 232-37
23. Bailey EV, Stone TW. The mechanism of action of amantadine in parkinsonism: a review. Arch Int Pharmacodyn 1976; 216: 246-62
24. Starr MS, Starr BS. Locomotor effects of amantadine in the mouse are not those of a typical glutamate antagonist. J Neural Transm Park Dis Dement Sect 1995; 9: 31-43
25. Atre-Vaidya N, Jampala VC. Electroconvulsive therapy in parkinsonism with affective disorder. Br J Psychiatry 1988; 152: 55-8
26. Golbe LI, Langston JW, Shoulson I. Selegiline and Parkinson's disease: protective and symptomatic considerations. Drugs 1990; 39: 646-51
27. Gerlach M, Riederer P, Youdim MBH. The molecular pharmacology of L-deprenyl. Eur J Pharmacol Mol Pharmacol 1992; 226: 97-108
28. Lees AJ, Parkinson's Disease Research Group of the United Kingdom. Comparison of therapeutic effects and mortality data of levodopa and levodopa combined with selegiline in patients with early, mild Parkinson's disease. BMJ 1995; 311: 1602-7
29. Dubois B, Montastruc JL. Association levodopa + selegiline et augmentation du risque de mortalité dans le traitement de la maladie de Parkinson. Rev Neurol Paris 1996; 152: 103-5
30. Montastruc JL, Chamontin B, Senard JM, et al. Pseudophaeochromocytoma in parkinsonian patient treated with fluoxetine plus selegiline. Lancet 1993; 341: 555
31. Fowler JS, Volkow ND, Logan J, et al. Monoamine oxidase B (MAO B) inhibitor therapy in Parkinson's disease: the degree and reversibility of human brain MAO inhibition by RO 19 632. Neurology 1993; 43: 1984-92
32. Montastruc JL, Baldy-Moulinier M, Lees AJ, et al. MDL 72,974 A: a new selective inhibitor of MAO B in Parkinson's disease [abstract]. New Trends Clin Neuropharmacol 1994; 8: 275
33. Mannisto PT, Kaakkola S. Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. Pharmacol Toxicol 1990; 66: 317-23
34. Mannisto PK. Clinical potential of catechol-O-methyltransferase (COMT) inhibitors as adjuvants in Parkinson's disease. CNS Drugs 1994; 1: 172-9
35. Merello M, Lees AJ, Webster R, et al. Effect of entacapone, a peripherally acting catechol-O-methyltransferase inhibitor, on the motor response to acute treatment with levodopa in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 1994; 57: 186-9
36. Limousin P, Pollak P, Pfefen JP, et al. Acute administration of levodopa-benserazide and tolcapone, a COMT inhibitor, in Parkinson's disease. Clin Neuropharmacol 1995; 18: 258-65
37. Blanchet PJ, Gomez-Mancilla B, Di Paolo T, et al. Is striatal dopaminergic receptor imbalance responsible for levodopa-induced dyskinesia? Fundam Clin Pharmacol 1995; 9: 434-42

38. Montastruc JL, Rascol O, Senard JM. Current status of dopamine agonists in Parkinson's disease management. Drugs 1993; 46: 384-93
39. Jenner P. The rationale for the use of dopamine agonists in Parkinson's disease. Neurology 1995; 45 Suppl. 3: S6-12
40. Montastruc JL, Rascol O, Rascol A. A randomized controlled study of bromocriptine versus levodopa in previously untreated parkinsonian patients: a 3 year follow-up. J Neurol Neurosurg Psychiatry 1989; 52: 773-5
41. Rinne UK. Early combination of bromocriptine and levodopa in the treatment of Parkinson's disease: a 5-year follow up. Neurology 1987; 37: 826-8
42. Montastruc JL, Rascol O, Senard JM, et al. A randomized controlled study comparing bromocriptine to which levodopa was later added with levodopa alone in previously untreated patients with Parkinson's disease: a five year follow-up. J Neurol Neurosurg Psychiatry 1994; 57: 1034-8
43. Lees AJ. Dopamine agonists in Parkinson's disease: a look at apomorphine. Fundam Clin Pharmacol 1993; 7: 121-8
44. Piccoli F, Riuggeri RM. Dopaminergic agonists in the treatment of Parkinson's disease: a review. J Neural Transm 1995; 45 Suppl.: 187-95
45. Rabey JM. Second generation of dopamine agonists: pros and cons. J Neural Transm 1995; 45 Suppl.: 213-24
46. Rascol O, Lees AJ, Senard JM, et al. A placebo-controlled study of ropinirole, a new D2 agonist, in the treatment of motor fluctuations of l-dopa-treated parkinsonian patients. Adv Neurol 1996; 69: 531-4
47. Sokoloff P, Martres MP, Schwartz JC. La famille des récepteurs de la dopamine. Med Sci 1993; 9: 12-20
48. Bedard PJ, Gomez-Mancilla B, Blanchet P, et al. Dopamine-receptor families and the treatment of Parkinson's disease. Clin Neuropharmacol 1995; 18 Suppl. 1: S178-87
49. Emre M, Rinne UK, Rascol A, et al. Effects of a selective partial D1 agonist CY 208-243, in de novo patients with Parkinson's disease. Mov Disord 1992; 7: 239-43
50. Montastruc JL, Fabre N, Blin O, et al. Does fluoxetine aggravate Parkinson's disease? A pilot prospective study [letter]. Mov Disord 1995; 10: 355-7
51. Bartholini G, Scatton B, Zivkovic B, et al. Gaba receptor agonists and extrapyramidal motor function: therapeutic implication for Parkinson's disease. Adv Neurol 1986; 45: 79-83
52. Rascol O, Fabre N, Blin O, et al. Naltrexone, an opiate antagonist, fails to modify motor symptoms in patients with Parkinson's disease. Mov Disord 1994; 9: 437-40
53. Narabayashi H, Kondo T, Yokichi F, et al. Clinical effects of L-threo-3,4-dihydroxyphenylserine in parkinsonism and pure akinesia. Adv Neurol 1986; 45: 593-602
54. Kaufman H, Oribe E, Yahr MD. Treatment of orthostatic hypotension with l-threo-3,4-dihydrophenylserine. In: Narabayashi H, Mizuno Y, editors. Norepinephrine deficiency and its treatment with l-threo-dops in Parkinson's disease and related disorders. London: Parthenon Publishing Group, 1993: 97-104
55. Colpaert FC. Noradrenergic mechanisms in Parkinson's disease: a theory. In: Briley M, Marien M, editors. Noradrenergic mechanism in Parkinson's disease. Boca Raton (FL): CRC Press, 1994: 225
56. Mavridis M, Degryse AD, Lategan AJ, et al. Effect of locus coeruleus lesion on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4 phenyl-1,2,3,6 tetrahydropyridine in monkeys: a possible role for the locus coeruleus in the progression of Parkinson's disease. Neuroscience 1991; 41: 507-23
57. Trendelenburg AU, Starke K, Limberger N. Presynaptic alpha 2A-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. Naunyn Schmiedebergs Arch Pharmacol 1994; 350: 473-81
58. Peyro-Saint-Paul H, Rascol O, Blin O, et al. A pilot study of idazoxan, an alpha2-antagonist in Parkinson's disease [abstract]. Therapie 1995; 50 Suppl.: S171
59. Greenamyre JT, O'Brien F. *N*-Methyl-D-aspartate antagonists in the treatment of Parkinson's disease. Arch Neurol 1991; 48: 977-81
60. Montastruc JL, Rascol O, Senard JM, et al. A pilot study of *N*-methyl-D-aspartate (NMDA) antagonists in Parkinson's disease. J Neurol Neurosurg Psychiatry 1992; 55: 630-1
61. Montastruc JL, Fabre N, Rascol O, et al. *N*-Methyl-D-aspartate (NMDA) antagonists and Parkinson's disease: a pilot study with dextromethorphan. Mov Disord 1994; 9: 242
62. Diederich N, Keipes M, Graas M, et al. La clozapine dans le traitement des manifestations psychiatriques de la maladie de Parkinson. Rev Neurol 1995; 151: 251-7
63. Rabey JM, Treves TA, Neufeld MY, et al. Low-dose clozapine in the treatment of levodopa-induced mental disturbances in Parkinson's disease. Neurology 1995; 45: 432-4
64. Tranchant C, Wees C, Warter JM. Maladie de Parkinson idiopathique et fonctions mitochondriales. Rev Neurol (Paris) 1995; 151: 157-60
65. Shoulson I. Neuroprotective clinical strategies for Parkinson's disease. Ann Neurol 1992; 32: S143-5
66. Birkmayer W, Knoll J, Riederer P, et al. Increased life expectancy resulting from addition of L-deprenyl to Madopar treatment in Parkinson's disease: a long term study. J Neurol Transm 1985; 7: 113-27
67. Tetrud JW, Langston B. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. Science 1989; 245: 519-22
68. Parkinson's Study Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. N Engl J Med 1993; 328: 178-83
69. Jankovic J. Neuroprotection: a reachable therapeutic goal? In: Stern BM, editor. Beyond the decade of the brain. Tunbridge Wells: Wells Medical Ltd, 1994: 109-27
70. Olanow CW, Hauser RA, Gauger L, et al. The effect of deprenyl and levodopa in the progression of Parkinson's disease. Ann Neurol 1995; 38: 771-7
71. Myllylä VV, Heinonen EH, Vuorinen JA, et al. Long-term effects of deprenyl in Parkinson's disease [abstract]. Mov Disord 1994; 9 Suppl. 1: 4
72. Brannan T, Yahr MD. Comparative study of selegiline plus L-dopa-carbidopa versus L-dopa-carbidopa alone in the treatment of Parkinson's disease. Ann Neurol 1995; 37: 95-8
73. Clow A, Hussain T, Glover V, et al. Pergolide can induce soluble superoxide dismutase in rat striata. J Neural Transm 1992; 90: 27-31
74. Felten DL, Felten SY, Fuller RW, et al. Chronic dietary pergolide preserves nigrostriatal neuronal integrity in aged-Fisher-344 rats. Neurobiol Aging 1992; 13: 339-51
75. Asanuda M, Ogawa N, Nishibayashi S, et al. Protective effects of pergolide on dopamine levels in the 6-hydroxydopamine-

lesioned mouse brain. Arch Int Pharmacodyn Ther 1995; 329: 221-30
76. Ogawa N, Tanaka K, Asanuma M, et al. Bromocriptine protects mice against 6-hydroxydopamine and scavenges hydroxyl free radicals *in vitro*. Brain Res 1994; 657: 207-13
77. Jenner P. Oxidative damage in neurodegenerative disease. Lancet 1994; 344: 796-8
78. Mizuno Y, Mori H, Kondo T. Potential of neuroprotective therapy in Parkinson's disease. CNS Drugs 1994; 1: 45-56
79. Takayama H, Ray J, Raymon HK, et al. Basic fibroblast growth factor increases dopaminergic graft survival and function in a rat model of Parkinson's disease. Nature Med 1995; 1: 53-8
80. Montastruc JL, Llau ME, Rascol O, et al. Drug-induced parkinsonism: a review. Fundam Clin Pharmacol 1994; 8: 293-306
81. Oertel WH. Parkinson's disease: epidemiology, (differential) diagnosis, therapy, relation to dementia. Arzneimittel Forschung 1995; 45: 386-9
82. Calne DB. Early detection of idiopathic parkinsonism. In: Sern MBMB, editor. Beyond the decade of the brain. Vol. 1. Tunbridge Wells: Wells Medical Ltd, 1994: 89-94

Correspondence and reprints: Professor *J.L. Montastruc*, Laboratoire de Pharmacologie Médicale et Clinique, INSERM U317, Faculté de Médecine, 37 allées Jules-Guesde, 31073 Toulouse Cedex, France.

EXHIBIT N

10.1

The Natural History of Parkinson's Disease

W. H. Poewe, MD, and G. K. Wenning, MD, PhD

There are still insufficient data on the natural course of Parkinson's disease (PD) owing to lack of standardized longitudinal follow-up studies. Reported progression rates in early PD vary considerably by a factor of 2 to 3. Similarly, data from sequential [^{18}F]dopa PET studies in PD patients have produced variable decline rates of PET indices ranging between 7 and 70% per decade. Risk factors for rapid progression include old age at onset, concomitant major depression, dementia, and akinetic–rigid symptom presentation. The introduction of levodopa into the routine treatment of PD patients had a dramatic impact on symptomatic control without affecting the underlying rate of disease progression. By contrast, monoamine oxidase (MAO) B inhibition by deprenyl monotherapy in early PD was shown to delay the need for levodopa by around 9 months. However, the neuroprotective action disappeared after 2 years of follow-up. Furthermore, deprenyl also failed to influence the subsequent development of levodopa-induced motor complications. Available studies on mortality in PD provide heterogeneous mortality rates, probably because of discrepancies between patient populations with respect to co-morbidity, disease stage at study entry, and diagnostic accuracy. However, the most recent follow-up from the DATATOP cohort suggests normal life expectancy in carefully selected patients without significant co-morbidity and with adequate treatment and expert follow-up.

Poewe WH, Wenning GK. The natural history of Parkinson's disease. Ann Neurol 1998;44(Suppl 1):S1–S9

Despite the many recent advances in the symptomatic treatment of Parkinson's disease (PD), there is still no realistic prospect for a cure. Although much effort has been directed toward the development of neuroprotective strategies that would modify the progression of disease, no candidate has yet emerged for which clinical studies have demonstrated any long-lasting and dramatic effect on the course of the illness. At the same time, there are still insufficient data concerning the natural course of the illness, because longitudinal follow-up observations according to modern standards were not possible in the pre-levodopa era. Current areas of uncertainty include the possible duration of a pre-clinical phase of PD, whether or not progression of clinically overt disease is linear or exponential, how to define and recognize distinct subtypes of the disease with different progression characteristics, and the risk factors and predictors for different rates of progression of disability. These are important issues to be addressed in attempting to design treatments that will hold or retard the underlying disease process. This article reviews some of the available evidence on the natural history of PD.

Progression of Motor Disability in Untreated PD

Hoehn and Yahr's clinical study of 856 patients with parkinsonism who were followed at the Columbia–Presbyterian Medical Center, which was published in 1967 just before the introduction of levodopa, provides important information regarding the natural history of untreated parkinsonism.[1] The authors rated the degree and progression of motor disability in a subgroup of 183 patients using the now well-established Hoehn–Yahr (HY) scale. Advanced disease corresponding to HY stages IV (severely disabling disease, patient still able to walk and stand unassisted but markedly incapacitated) and V (confinement to bed or wheelchair unless aided) was documented in 16% of patients with a disease duration of less than 5 years, increasing to 37% and 42% of patients with disease durations up to 10 and 15 years, respectively. Median delays before reaching HY stages IV and V were 9.0 ± 7.2 and 14.0 ± 3.4 years, respectively (Table 1). Although more severe HY stages were associated with longer duration of disease, there was a remarkable variation of progression of disability. Hoehn and Yahr observed that 26 of 70 (37%) patients whose illness had been present less than 5 years had reached stage III or beyond. Conversely, of all patients with a duration of illness 10 years or longer, 20 of 59 (34%) were still in stages I and II. These findings suggest heterogeneity of disease progression in PD. However, the patients of this early series were not diagnosed according to validated clinical criteria, and a considerable proportion of patients with atypical parkinsonism may therefore have been included. Hoehn and Yahr also determined the extent of motor disability and death in a longitudinal

From the Department of Neurology, University Hospital, Innsbruck, Austria.

Address correspondence to Dr Poewe, Department of Neurology, University Hospital, Anichstrasse 35, 6020 Innsbruck, Austria.

Table 1. Progression of PD

Reference	HY Stage I	HY Stage II	HY Stage III	HY Stage IV	HY Stage V
1[a]	3.0 ± 7.1	6.0 ± 6.9	7.0 ± 6.3	9.0 ± 7.2	14.0 ± 3.4
2[b]	Not available	2.9 ± 0.2	5.5 ± 0.3	7.5 ± 0.6	9.7 ± 1.0

[a]*Median* disease durations (±1 SD) at successive Hoehn and Yahr (HY) stages in years.
[b]*Mean* disease durations (±1 SD) at successive Hoehn and Yahr (HY) stages in years.

follow-up survey of 271 patients. Almost one-third (28%) of patients became severely disabled or died within 5 years of disease onset. This ratio rose to 61% (up to 10 years of disease duration) and 90% (more than 15 years of disease duration).

Similar findings were subsequently reported in 1977 by Martilla and Rinne,[2] who studied the disability and progression of PD in southwest Finland, including 91% of all known cases in this area. Patients with unilateral parkinsonism (HY stage I) developed bilateral features after a mean of 2.9 years. The HY stages III, IV, and V were each reached after a mean of 2 to 2.5 years, with stage V occurring 10 years after disease onset (Table 1). The time intervals between the different HY stages showed marked variation throughout the entire disease course, eg, from 0 to 30 years for bilateralization of parkinsonism (HY stage I to stage II). The speed of progression remained rather constant for individual patients. There was a correlation of the time interval between HY stages I and II and progression to advanced disability (HY stages III–V).

Taken together, these findings from the prelevodopa era seem to suggest a rather unfavorable course of PD, with a mean delay of 10 to 15 years before patients become severely disabled or die. At the same time, the data point to marked heterogeneity of disease progression, which is not likely to be completely accounted for by lack of strict diagnostic criteria in these surveys.

Somatotopic Progression of PD

It is generally accepted that progression of motor symptoms in PD reflects advancing nigral pathology.[3] Neuron loss within the substantia nigra in PD occurs in an uneven fashion, with neurons in the ventrolateral tier being most severely depleted.[4,5] Projections of substantia nigra neurons to the patch areas of the putamen mainly arise in the ventral tier, with a mediolateral topographic distribution of nigroputaminal patch input.[6] A distinct rostrocaudal and mediolateral distribution of corticoputaminal motor projections was demonstrated autoradiographically in primates.[7] The foot field extends rostrodorsally and the arm fields caudoventrally in the putamen. Kish et al.[8] found an uneven pattern of dopamine loss in the striatum of human PD patients, with pronounced loss in the dorsal and caudal parts of the putamen. On the basis of this finding, they concluded that a disturbance of motor control in the leg should be the first clinical sign of PD. Similar suggestions were made in a study of somatotopic progression of levodopa-induced dyskinesias.[9]

We have examined aspects of somatotopic disease progression in a retrospective study involving 253 patients with a clinical diagnosis of PD, according to established criteria, who underwent routine treatment and follow-up at our Movement Disorder Clinic over a 10-year period. Initial symptoms were reported by the patients and/or their relatives at the first visit and corroborated by neurologic examination. Six-monthly outpatient visits over the entire follow-up period included ratings on the Columbia Unified Rating Scale before 1986 and the Unified Parkinson's Disease Rating Scale thereafter. As expected, tremor was the most common initial feature, constituting the presenting complaint in 63% of patients. There was an unexpected laterality of the presenting symptoms, with initial complaints more often starting on the right compared to the left side (61% vs 39%). In patients with unilateral arm involvement, spread of symptoms to the homolateral leg occurred between 0.8 and 1.4 years from onset, whereas contralateral spread, corresponding to the transition from HY stage I to stage II, had slightly longer delays of 2.1–3.4 years (Fig 1).

Interestingly, James Parkinson in his original monograph[10] noted similar latencies for bilateral progression of symptoms when he described his case 6: "About eleven or twelve, or perhaps more, years ago, he first perceived weakness in the left hand and arm, and soon after found the trembling commence. In about three years afterwards the right arm became affected in a similar manner . . . ."

The fact that ipsilateral progression of symptoms occurred before first signs appeared contralaterally might indicate accelerated progression of nigral pathology once the symptomatic threshold has been reached compared to the rate of presymptomatic nigral cell loss. The unexpected finding of preferential right-sided over left-sided presentation is difficult to explain, and further studies of somatotopic disease progression in the initial phase of PD appear to be warranted. In keeping with the notion of unilateral or asymmetrical disease

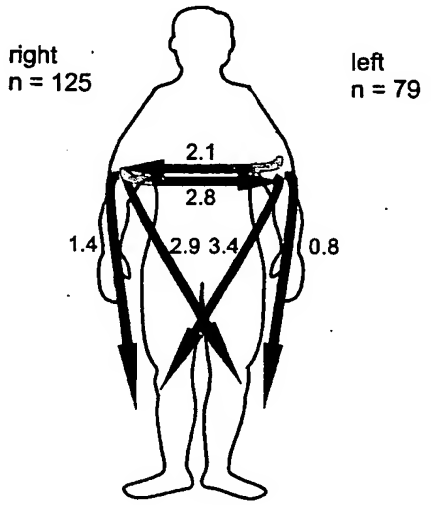


Fig 1. Somatotopic progression of parkinsonian features in patients with disease onset in either right or left upper limb. Mean latencies (years) to involvement of second extremity are shown.

onset, longitudinal follow-up studies suggest that one side of the body tends to be affected predominantly and persistently throughout the course of illness.[11]

Rates of Progression in Early PD

The concept of neuroprotective treatment in PD has prompted careful monitoring of clinical indices of disease progression in studies of putative neuroprotective agents. The largest of these trials, the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP)[12–16] was a controlled clinical trial designed to examine the effects of deprenyl and tocopherol in 800 patients with early PD (HY stages I and II, <5 years disease duration) who were not receiving or requiring any antiparkinsonian medications. By use of a 2 × 2 factorial design, patients were assigned randomly to either placebo, deprenyl (10 mg/day), tocopherol (2,000 IU/day), or both, and were followed to determine if and when levodopa treatment was required (primary end point). On the basis of DATATOP study results, it is possible to determine average annual rates of decline of motor function in 353 patients with levodopa-naive PD (placebo or tocopherol plus placebo treatment) over a mean follow-up period of 16 ± 6 months. Of these patients, 120 did not reach the end point and were followed for a mean of 21 ± 4 months ("survivors"). Annual rates of decline in motor function are shown in Fig 2. Linear extrapolation of these progression rates would lead to severe disability over a period of 10 years, similar to earlier observations in untreated parkinsonism.[1] In contrast, in the DATATOP survivor groups who had not reached the primary end point, ie, motor disability requiring levodopa substitution, annual progression of motor disability, as measured by UPDRS part III scores, was markedly slower compared to the total patient cohort (Fig 2).

Similar observations have been made regarding changes in the UPDRS score followed over a treatment period of 12 months in 101 patients with untreated early PD who were receiving treatment with levodopa or bromocriptine combined with placebo or deprenyl in a four-arm treatment study.[17] If deprenyl patients are excluded for possible neuroprotective effects of this drug, the calculated annual rates of decline in UPDRS motor scores are on the order of 4% of the maximum score value in bromocriptine- or levodopa-treated patients, similar to the more slowly progressing survivors of the DATATOP study.

Slower rates of motor deterioration were observed in a clinical study of 238 patients with PD who were treated with levodopa and/or bromocriptine, a small number also receiving deprenyl.[18] The investigators restricted their analysis to UPDRS bradykinesia subscores during defined "off" periods (10–12 hours after stopping all antiparkinsonian drugs). Given an average disease duration of 8.3 ± 5.8 years, the rate of progression of bradykinesia was 0.118 per year, corresponding

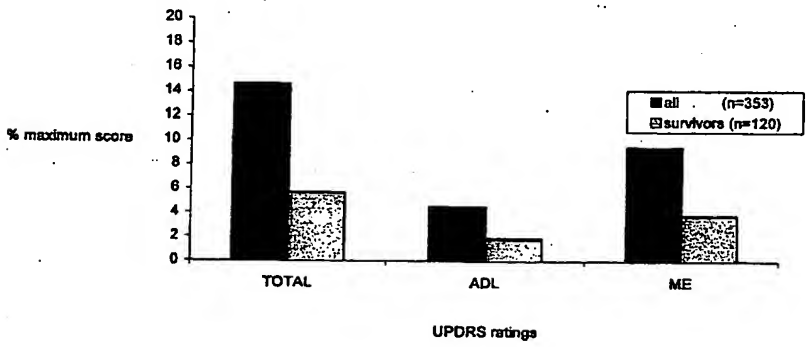


Fig 2. Annual rates of decline of Unified Parkinson's Disease Rating Scale (UPDRS) subscores in early PD. Data derived from the Parkinson Study Group.[13] ADL = activities of daily living (UPDRS part I); ME = motor examination (UPDRS part II); all = levodopa-naive treated with placebo or placebo plus tocopherol; survivors = patients not requiring levodopa at end point of study.

to 0.49% of the maximum attainable bradykinesia score of 24. However, as a function of duration, the bradykinesia score initially advanced rapidly, with an annual rate of decline of 3.5% in the first year, which fell to 1.5% in the tenth year after symptom onset. The available studies therefore indicate that the annual rate of progression of motor disturbance during the early stages of the disease may vary by a factor of 2 to 3.

It is intriguing that annual rates of decline of clinical motor indices were considerably slower in two studies that examined patients receiving dopaminergic therapy[17,18] compared to the speed of progression of untreated patients from the DATATOP trial, at least if one looks at those who reached the end point.[13] Whether or not this indicates some "neuroprotective" effect of dopamine replacement is open to speculation.

Furthermore, most of the clinical evidence suggests that progression of motor disability may be more rapid early in the disease compared to the later stages. In the seminal Hoehn and Yahr study,[1] short symptom duration of less than 4 years was most commonly observed in stages I–III, and with each progressive stage of severity the proportion of patients with longer disease duration grew. Similarly, Bonnet et al.,[19] in a cross-sectional study of 193 PD inpatients, found that most of the disease progression occurred in the first 9 years of disease. Basal scores after drug withdrawal increased steadily up to a disease duration of 9 years and showed little change thereafter.

In accordance with such clinical observations, Morrish and colleagues,[20] using sequential [^{18}F]dopa PET scans in two groups of patients with short and long disease duration, found evidence for a faster annual rate of reduction of the putamen (K_i) in patients with recent onset compared to those with longer duration of disease.

Rates of Nigral Cell Loss and Decline of Striatal [^{18}F]Dopa Uptake in PD

It is widely accepted that the severity of motor symptoms in PD is closely related to the degree of nigral cell depletion and associated striatal dopamine loss. Accordingly, progression of motor disability should reflect rates of decline in number of substantia nigra neurons. According to a hypothesis originally put forward by Calne and Langston,[21] the progression of PD should follow the time course of age-related nigral neuronal attrition. The latter has been estimated to occur at a rate of close to 5% per decade in the UK Parkinson's Disease Society Brain Bank study by Fearnley and Lees,[5] which included 36 control brains from non-parkinsonian subjects aged 29–91 years at death. However, it appears difficult to account for the substantial variability in rates of clinical progression of PD if aging is assumed to be the primary mechanism that accounts for the time course of functional decline. Fearnley and Lees[5] showed that the regional distribution of nigral cell loss observed with normal aging is fundamentally different from that in patients with various stages of PD. Whereas the lateral ventral tier of the substantia nigra was relatively spared in control subjects, it was most severely affected in PD patients. This study demonstrated a good correlation between duration of disease and degree of nigral neuron loss. Decline of total and regional neuron counts increased exponentially with disease duration. In the first decade after disease onset, Fearnley and Lees[5] noted a 45% decrease in nigral cell counts, 10-fold greater than the rate of loss that could be accounted for by aging. Again, this postmortem study supported clinical observations of a more rapidly advancing disease early in the course compared to later years.

More recently, [^{18}F]dopa PET measurements have been used as an in vivo approach to measurement of rates of progression of the underlying pathology in sequential studies in PD patients. Although it is well established that putaminal [^{18}F]dopa uptake is correlated with clinical severity of symptoms,[20] the evidence that [^{18}F]dopa PET indices are a reflection of nigral cell count is far from certain. It is therefore possible that rates of decline of [^{18}F]dopa putaminal uptake in PD represent a more sensitive and objective means of studying progression of striatal dopaminergic dysfunction but do not actually reflect advancing neuronal attrition in the substantia nigra.[22]

Data from sequential [^{18}F]dopa PET studies in PD patients have produced conflicting results. Vingerhoets and colleagues[3] studied 16 PD patients and 10 control subjects, with an interstudy interval of 7 years. The striatal:occipital ratio declined by 7.8% per decade in patients compared to 3% in healthy controls, corresponding to an annual decline of striatal dopaminergic metabolic function of 0.78% of the normal mean. In contrast, Morrish and colleagues[20] reported a much more rapid progression of PET indices in a sequential study of 10 patients with recent-onset PD with a mean duration of 18 months and in seven patients with a longer disease duration of 71 months. Interstudy intervals were 15 and 18 months, and comparisons were made with 10 normal subjects who were scanned twice with a mean interval of 32 months. In this study, the average left to right putaminal [^{18}F]dopa influx constant (K_i) was chosen as the most consistent and reliable measurement of striatal metabolism. The K_i declined at an annual rate of 7% of the normal mean in both patient groups taken together.

Preclinical Disease and Symptomatic Threshold

A number of postmortem studies have attempted to estimate symptomatic thresholds of nigral cell loss be-

yond which parkinsonian symptoms would become clinically manifest. In an early study by Bernheimer and colleagues[23] the degree of reduction in mean striatal dopamine content in patients with a mean duration of symptoms of more than 9 years led the authors to estimate that nigral cell count was likely to be reduced by at least 50% at disease onset. Fearnley and Lees[5] calculated a symptomatic threshold of nigral cell loss of 69% of normal. Similar figures were reported by Paulus and Jellinger[24] for the lateral substantia nigra. These authors also found an early exponential attrition of nigral neurons, from which they concluded a short duration of a preclinical phase of around 4.7 years. Assuming a linear progression of PET indices, Morrish and colleagues[20] calculated a similar duration of a possible preclinical period of PD in their sequential [^{18}F]dopa study of PD. The same assumption led to a considerably longer estimate in the study by Vingerhoets et al.,[3] with a possible preclinical period of PD of several decades. A similarly long latent phase of the illness had also been postulated by Gibb et al.[25] when age-specific prevalence rates of incidental Lewy bodies in the substantia nigra were compared with those of age-specific prevalence rates of clinical PD. Furthermore, concordance of monozygotic twins may require more than 20 years to become manifest, which could also be regarded as evidence for a long preclinical stage of PD.[26]

Gonera et al.[27] have recently presented evidence for a prodromal phase of PD when health records of 60 patients with PD and 58 matched controls were compared over the decade before onset of classical PD. Prodromal symptoms occurring significantly more often in the pre-PD group compared to controls included mood changes, limb pain, paresthesias, and hypertension. The duration of this prodromal phase was between 4 and 6 years.

Although the exact duration of a latent phase of PD therefore remains controversial, there is little doubt about its existence, and it is the prime target period for neuroprotective treatment. Whereas both [^{18}F]dopa PET and β-CIT SPECT scanning can detect presymptomatic nigrostriatal dopaminergic function, a widely applicable screening test for preclinical PD is still lacking.

Impact of Drug Treatment on Disease Progression

It is widely accepted that the introduction of levodopa into the routine treatment of patients with PD had a dramatic impact on the progression of disability and, at least initially, on the mortality of this disorder. When disease progression and mortality in 282 patients with PD receiving levodopa treatment is compared with a cohort of patients from the pre-levodopa area, Hoehn[28] found that levodopa-treated patients remained for 3 to 5 years longer in each stage of the HY scale compared to untreated patients. This is in contrast to patients with levodopa-unresponsive parkinsonism, such as multiple-system atrophy, whose progression of disability corresponds to that of untreated PD.[29] Hoehn[28] also compared a number of patients who had died or had become severely disabled for successive 5-year periods up to disease durations of 15 years. In levodopa-treated patients, the percentage of those disabled or dead in each epoch of disease duration was reduced by 30 to 50%. However, there was no detectable difference between treated and untreated patients in the actual rate of disease progression.

More recently, drug development has focused on agents that might modify the natural progression of PD. Such neuroprotective properties have been debated with respect to antioxidants including vitamin C, tocopherol, and deprenyl, as well as antiglutamate agents including amantadine, dopamine agonists, and neuronal growth factors. Thus far deprenyl is the only drug with some potential to modify rates of progression in early PD in properly controlled clinical trials. The available studies have provided evidence that (a) deprenyl treatment of untreated patients with early PD may delay the need for levodopa by around 9 months and reduce the annual rates of decline of motor scores in the first 12 months of treatment by about 50%[12,13,30]; (b) its modifying effect on disease progression is not sustained beyond 2 years of treatment[14]; (c) prior treatment with deprenyl does not appear to influence the subsequent response to levodopa, including the development of motor fluctuations and dyskinesias[15]; and (d) co-treatment with deprenyl is associated with a long-lasting reduction in daily levodopa requirements for follow-up periods of up to 5 years.[31] However, despite the initially encouraging results obtained with deprenyl there is as yet no treatment with a proven substantial impact on the clinical rate of progression of PD.

Factors Influencing Disease Progression

Several authors have observed a less rapidly advancing disease in tremor dominant cases. For example, Martilla and Rinne,[2] found that patients presenting predominantly with tremor carried a slightly better prognosis than those presenting with any other parkinsonian sign. Seventy percent of those patients starting with tremor remained in stages I and II compared to 60% of those starting with other symptoms. A later study confirmed the more benign nature of tremor-dominant PD and observed a correlation between tremor and preserved mental state, early age at onset,

positive family history, and better prognosis.[32] Similar observations were also reported by Roos et al.[33]

In three recent clinical studies, age at onset emerged as an important factor determining disease progression.[34–36] Rapid disease progression has been recognized in elderly PD patients and may be associated with increasing levodopa-unresponsive axial motor disability, such as gait unsteadiness, freezing, and dysarthria.[34–36] Although Lewy body pathology in substantia nigra appears to be similar in young- versus old-onset patients,[37] there is a striking difference in the prevalence of dementia between these groups of patients. Among several large series in patients with young-onset (less than 40 years of age) PD of long duration (approximately 15 years) and advancing degree whose mean age was below 60 years, dementia was vanishingly rare.[38–40]

Cognitive decline has been shown to be associated with more rapid progression of disability in PD. Biggins et al.[41] used serial assessments of cognition, mood, and parkinsonian disability over 54 months in a cohort of 87 PD patients and a control group of 50 matched healthy individuals. Dementia (DSM III-R) was observed in 6% of the patients at study entry and in 25% at the end of follow-up, yielding a 19% incidence, whereas none of the controls developed dementia. Patients who became demented were older, had a longer duration of disease, were more likely to be male, and were disabled by PD at entry. PD patients with hallucinations related to dementia are at high risk of dying in nursing homes within few years after placement.[42,43]

Dementia may be linked to certain clinical subtypes of PD. In a series of 155 PD patients, severe dementia was recorded in 8%. Almost half of the demented patients exhibited a predominantly akinetic–rigid syndrome, as opposed to 19% of cases in the non-demented group. Tremor-dominant cases were not observed among the demented patients who were older and had a shorter disease duration than the non-demented.[44] Starkstein et al.[45] reported more severe disability as well as considerable cognitive decline in PD patients with major depression at study entry compared to patients with no or minor depression, indicating that mood disorder may be another risk factor for progression of disability.

Mortality

In Hoehn and Yahr's series of 672 patients with primary parkinsonism, 44% had died during the follow-up period, yielding a mortality ratio of 2.9 times that expected in the age-matched population. Average age at death was 67 years and the average duration of life after diagnosis was 9.4 (1–33) years.[1] This was recently confirmed in a community-based study of 467 elderly subjects, 42% of whom exhibited parkinsonian signs.[46] Over a mean follow-up of 9 years, 78% of these individuals with parkinsonism had died. Adjusted for age and sex, their overall risk for death was 2.0 times that of persons without parkinsonism in the same cohort. Gait disorder was most closely associated with increased mortality in the parkinsonian subjects.

However, the Columbia–Presbyterian experience may not have been representative of the entire parkinsonian population. Nobrega et al.[47] found the mortality to be increased by 1.6-fold in parkinsonian patients in Rochester, Minnesota, between 1935 and 1966. An earlier analysis of this same population by Kurland[48] had found an even lower excess mortality of 1.41. The difference between clinic surveys such as the study by Hoehn and Yahr and population surveys such as those performed in Rochester, Minnesota, should be kept in mind when attempts are made to compare studies.

The introduction of levodopa had a striking effect on the excess mortality observed in untreated patients with PD. Hoehn[28] reported a mortality ratio of 1.5 in 182 patients seen within 2 years of disease onset after 1968, when levodopa became available. Hence, compared to the 1967 study mortality was reduced by half. Hoehn suggested that the life expectancy with parkinsonism was close to that of the general population. Similar mortality ratios in treated patients were reported by Yahr[49] and Shaw et al.[50] Diamond and colleagues[51] have indicated that mortality in levodopa-treated patients is positively correlated with the delay before starting treatment. Several studies have shown that the effects of levodopa on mortality are apparent in the early years of the disease but that mortality rises later in the disease course despite levodopa therapy.[51–53]

However, all but one survival study in patients with parkinsonism have recruited their patients from specialist centers, and only three studies have compared the mortality of parkinsonian patients with matched control subjects.[54–56] Ben Shlomo and Marmot[56] reported a mortality ratio of 2.6 in their cohort of 220 parkinsonian patients, controlling for age, sex, and geographic region. Both ischemic heart disease and cerebrovascular disease were associated with significantly elevated hazard ratios. However, because their patients were recruited between 1970 and 1972, not all of them would have received optimal dopaminergic substitution during the early stages of the illness, perhaps accounting for some of the excess mortality.

In a recent analysis of mortality among the 800 patients with early PD enrolled in the DATATOP trial, the overall death rate was 17.1% over 8.2 years of observation, corresponding to 2.1% per year.[16] The mortality rate was substantially lower than that reported in most of the previous studies and was even lower than the expected mortality rate of an age-matched United States population not affected by PD. Increased life ex-

pectancy due to deprenyl treatment has been reported by Birkmayer et al.[57] but the DATATOP mortality rate was unaffected by deprenyl, tocopherol, or combined treatment.

In contrast to these findings, the Parkinson's Disease Research Group (PDRG) of the United Kingdom reported increased mortality associated with deprenyl treatment when given as adjunct to levodopa in an open-label study in patients already disabled at entry.[31] A total of 782 patients were enrolled between 1982 and 1990 and were randomized to one of three treatment arms: (a) standard levodopa treatment; (b) standard levodopa and deprenyl; or (c) bromocriptine. The average follow-up for the study of mortality was 5.6 years, and data have been reported only for arms 1 and 2. The ratio of mortality in arm 2 (levodopa with deprenyl) compared with arm 1 (levodopa alone) was 1.57, and the difference in survival between the two arms was significant. Therefore, mortality among deprenyl-treated patients was 60% higher than that in patients without deprenyl. There was no obvious explanation for this finding. However, in contrast to the DATATOP trial, the United Kingdom study was characterized by its open-label design and also its recruitment of slightly older, more disabled subjects requiring symptomatic antiparkinsonian therapy. In addition, there has been major criticism of its methodology.[58] In the recent SINDEPAR study, 101 untreated PD patients were randomized to receive deprenyl or placebo followed by symptomatic treatment with either levodopa or bromocriptine.[17] After a mean follow-up of 4.5 years there were six (12%) deaths in the placebo group and three (6%) deaths in the deprenyl group. Although this difference was not significant because of relatively small patient numbers, Olanow et al.[58] concluded that there was certainly no increased mortality in deprenyl-treated patients. Finally, there are seven other controlled long-term trials of PD patients treated with deprenyl and/or levodopa who have been followed for a minimum of 3.5 years.[59] Deaths were observed in 5.2% of 524 deprenyl-treated patients and in 5.5% of 658 non–deprenyl-treated patients. These differences were again not statistically significant.

Available studies on the mortality in PD therefore provide confusingly heterogeneous mortality ratios ranging from below 1 to threefold that of a standard population. These conflicting data are probably due to wide discrepancies between patient populations studied with respect to co-morbidity, stage of disease at entry, and diagnostic accuracy. The most recent data from the DATATOP cohort suggest, however, that carefully selected patients with early and "pure" PD without significant co-morbidity have "supernormal" life expectancy when adequately treated and regularly followed at specialist centers. The impact of levodopa, although beneficial in the early years of treatment, does not appear to affect mortality in the long term. Likewise, contrary to earlier claims, deprenyl treatment is not associated with increased life expectancy in PD, but there is also no convincing evidence that it contributes to excess mortality.

Conclusions

Despite all recent attempts at developing neuroprotective treatments, PD remains a relentlessly progressive disorder. Available clinical data, however, suggest that PD exhibits considerable heterogeneity when indices of progression and mortality are studied. Although a number of modifying factors have been identified clinically, it is worthwhile to try to define subgroups of PD with different progression characteristics. It may well be that, similar to other disorders, any preventive neuroprotective strategy may have an effect restricted only to certain subsets of patients and that such effects may get lost when cohorts with different underlying characteristics of progression are studied.

We thank Dr L. Schelosky for analyzing the data on somatotopic disease progression and Dr M. Gollner for preparing Fig 2.

References

1. Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. Neurology 1967;17:427–442
2. Marttila PJ, Rinne UK. Disability and progression in Parkinson's disease. Acta Neurol Scand 1977;56:159–169
3. Vingerhoets FJG, Snow BJ, Lee CS, Schulzer M, Mak E, Calne DB. Longitudinal fluorodopa positron emission tomographic studies of the evolution of idiopathic parkinsonism. Ann Neurol 1994;36:759–764
4. Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature 1988;334:345–348
5. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991;114:2283–2301
6. Gerfen CR, Herkenham M, Thibault J. The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. J Neurosci 1987;7:3915–3934
7. Künzle H. Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in *Macaca fascicularis*. Brain Res 1975;88: 195–209
8. Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease: pathophysiological and clinical implications. N Engl J Med 1988;318:876–880
9. Vidailhet M, Bonnet AM, Marconi R, Guider-Khouja N, Agid Y. Do parkinsonian symptoms and levodopa-induced dyskinesias start in the foot? Neurology 1994;44:1613–1616
10. Parkinson J. An essay on the shaking palsy. London: Sherwood, 1817

11. Lee CS, Schulzer M, Mak E, et al. Patterns of asymmetry do not change over the course of idiopathic parkinsonism: implications for pathogenesis. Neurology 1995;45:435–439
12. The Parkinson Study Group. DATATOP: a multicenter controlled clinical trial in early Parkinson's disease. Arch Neurol 1989;46:1052–1060
13. The Parkinson Study Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. N Engl J Med 1993;328:176–183
14. Parkinson Study Group. Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP subjects not requiring levodopa. Ann Neurol 1996;39:29–36
15. Parkinson Study Group. Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP subjects requiring levodopa. Ann Neurol 1996;39:37–45
16. The Parkinson Study Group. Mortality in DATATOP: a multicenter trial in early Parkinson's disease. Ann Neurol 1998;43: 318–325
17. Olanow CW, Hauser RA, Gauger L, et al. The effect of deprenyl and levodopa on the progression of Parkinson's disease. Ann Neurol 1995;38:771–777
18. Lee CS, Schulzer M, Mak K, et al. Clinical observations on the rate of progression of idiopathic parkinsonism. Brain 1994;117: 501–507
19. Bonnet AM, Loria Y, Saint-Hilaire MH, Lhermitte F, Agid Y. Does long-term aggravation of Parkinson's disease result from nondopaminergic lesions? Neurology 1987;37:1539–1542
20. Morrish PK, Sawle GV, Brooks DJ. An [^{18}F]dopa-PET and clinical study of the rate of progression in Parkinson's disease. Brain 1996;119:585–591
21. Calne DB, Langston JW. Aetiology of Parkinson's disease. Lancet 1983;2:1457–1459
22. Morrish PK. Parkinson's disease is not a long-latency illness. Mov Disord 1997;12:849–854
23. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 1973;20:415–455
24. Paulus W, Jellinger K. The neuropathologic basis of different clinical subgroups of Parkinson's disease. J Neuropathol Exp Neurol 1991;506:743–755
25. Gibb WRG, Lees AJ. The relevance of the lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745–752
26. Duvoisin RC. The genetics of Parkinson's disease: a review. In: Rinne UK, Nagatsu T, Horowski R, eds. International Workshop Berlin Parkinson's Disease. Bussum, NL: Medicom, 1991: 38–57
27. Gonera EG, van't Hof M, Berger HJC, van Weel C, Horstink MWIM. Symptoms and duration of the prodromal phase in Parkinson's disease. Mov Disord 1997;12:871–876
28. Hoehn MM. Parkinsonism treated with levodopa: progression and mortality. J Neurol Transmiss 1983;(Suppl 19):253–264
29. Wenning GK, Ben Shlomo Y, Magalhaes N, Daniel SE, Quinn NP. Clinical features and natural history of multiple system atrophy: an analysis of 100 cases. Brain 1994;117:835–845
30. Tetrud JW, Langston JW. The effect of deprenyl (selegeline) on the natural history of Parkinson's disease. Science 1989;245: 519–522
31. Lees AJ (Parkinson's Disease Research Group of the United Kingdom). Comparison of therapeutic effects and mortality data of levodopa and levodopa combined with selegeline in patients with early, mild Parkinson's disease. BMJ 1995;311: 1602–1607
32. Zetusky WJ, Jankowic J, Pirozzolo FJ. The heterogeneity of Parkinson's disease: clinical and prognostic implications. Neurology 1985;35:522–526
33. Roos RAC, Jongen CF, van der Velde EA. Clinical course of patients with idiopathic Parkinson's disease. Mov Disord 1996; 3:236–242
34. Goetz CG, Tanner CM, Stebbins GT, Buchman AS. Risk factors for progression in Parkinson's disease. Neurology 1988;38: 1841–1844
35. Blin J, Dubois B, Bonnet AM, Vidailhet M, Brandabur M, Agid Y. Does ageing aggravate parkinsonian disability? J Neurol Neurosurg Psychiatry 1991;54:780–782
36. Diamond SG, Markham CH, Hoehn MM, McDowell FH, Muenter MD. Effect of age at onset on progression and mortality in Parkinson's disease. Neurology 1989;39:1187–1190
37. Gibb WRG, Lees AJ. A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. Neurology 1988;38:1402–1406
38. Gershanik OS, Leist A. Juvenile Parkinson's disease. Adv Neurol 1986;45:213–216
39. Lima B, Neves G, Nora M. Juvenile parkinsonism: clinical and metabolic characteristics. J Neurol Neurosurg Psychiatry 1987; 50:345–348
40. Quinn N, Critchley P, Marsden CD. Young onset Parkinson's disease. Mov Disord 1987;2:73–91
41. Biggins CA, Boyd JL, Harrop FM, et al. A controlled, longitudinal study of dementia in Parkinson's disease. J Neurol Neurosurg Psychiatry 1992;55:566–571
42. Goetz CG, Stebbins GT. Risk factor for nursing home placement in advanced Parkinson's disease. Neurology 1993;43: 2227–2229
43. Goetz CG, Stebbins GT. Mortality and hallucinations in nursing home patients with advanced Parkinson's disease. Neurology 1995;45:669–671
44. Gerstenbrand F, Poewe WH. The classification of Parkinson's disease. In: Stern GM, ed. Parkinson's disease. London: Chapman and Hall, 1987:315–331
45. Starkstein SE, Mayberg HS, Leiguarda R, Preziosi TJ, Robinson G. A prospective longitudinal study of depression, cognitive decline, and physical impairments in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 1992;55:377–382
46. Bennett DA, Beckett LA, Murray AM. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med 1996;334:71–76
47. Nobrega FT, Glattre E, Kurland LT, Okazaki H. Comments on the epidemiology of parkinsonism including prevalence and incidence statistics for Rochester, Minnesota, 1935–1966. In: Barbeau A, Brunette JR, eds. Progress in neurogenetics. Amsterdam: Excerpta Medica, 1967:474–485
48. Kurland LT. Epidemiology: Incidence, geographic distribution and genetic considerations. In: Field WJ, ed. Pathogenesis and treatment of parkinsonism. Springfield, IL: Charles C. Thomas, 1958:5–49
49. Yahr MD. Evaluation of long-term therapy in Parkinson's disease: mortality and therapeutic efficacy. In: Birkmayer W, Hornykiewicz O, eds. Advances in parkinsonism. Basel: Editiones Roche, 1976:435–444
50. Shaw KM, Lees AJ, Stern GM. The impact of treatment with levodopa on Parkinson's disease. Q J Med 1980;49:283–293
51. Diamond SG, Markham CH, Hoehn MM, McDowell FI, Muenter MD. Multicenter study of Parkinson mortality with early versus later dopa treatment. Ann Neurol 1987;22:8–12
52. Curtis L, Lees AJ, Stern GM, Marmot MG. Effect of L-dopa on the course of Parkinson's disease: Lancet 1984;2:211–212
53. Uitti RJ, Ahlskog JE, Maraganore DM, et al. Levodopa therapy

and survival in idiopathic Parkinson's disease: Olmstead County Project. Neurology 1993;43:1918–1926
54. Ebmaier YP, Calder SA, Crawford JR, Stewart L, Besson JAO, Mutch WJ. Mortality and causes of death in idiopathic Parkinson's disease: results from the Aberdeen whole population study. Acta Neurol Scand 1990;81:294–299
55. Rajput AH, Offord KP, Beard CU, Kurland LT. Epidemiology of parkinsonism: incidence, classification and mortality. Can J Neurol Sci 1992;19:103–107
56. Ben Shlomo Y, Marmot MG. Survival and cause of death in a cohort of patients with parkinsonism: possible clues to aetiology. J Neurol Neurosurg Psychiatry 1995;58:293–299
57. Birkmayer W, Knoll J, Riederer P, et al. Increased life expectancy resulting from addition of l-deprenyl to Madopar treatment of Parkinson's disease: a long-term study. J Neural Transmiss 1985;64:113–127
58. Olanow CW, Fahn S, Langston JW, Godbold J. Selegiline and mortality in Parkinson's disease. Ann Neurol 1996;40: 841–845
59. Maki-Ikola O, Kilkku O, Heinonen E. Other studies have not shown increased mortality [letter]. BMJ 1996;312:702

EXHIBIT O

Clinical review

Science, medicine, and the future

Parkinson's disease

A H V Schapira

Parkinson's disease is the commonest neurodegenerative disease after Alzheimer's disease, with an estimated incidence of 20/100 000 and a prevalence of 150/100 000. It is characterised clinically by asymmetric onset of bradykinesia, rigidity, and, usually, resting tremor. The cause of the most common clinical features is the death of dopaminergic neurones in the substantia nigra of the midbrain. Lewy bodies are present in a proportion of surviving neurones. At the pathological level there is overlap with other neurodegenerative disorders including Alzheimer's disease, and this has been used to support the view that these diseases may share some common pathogenetic mechanisms.

Parkinson's disease causes substantial morbidity and results in a shortened life span. It also has considerable economic consequences, including loss of earnings, cost of care, and cost of drug treatment (currently calculated at $1.1bn (£700m) worldwide). A major problem for researchers and clinicians is that, by the time patients' symptoms become sufficiently apparent for them to seek help, about 70-80% of their dopaminergic neurones may have already died. The length of the presymptomatic phase or incubation time of the disease may vary depending on the cause (fig 1). The main challenges in the treatment of Parkinson's disease are therefore (*a*) to protect dopaminergic neurones so that either the disease is prevented or its progression is slowed and (*b*) to provide treatment early to "rescue" neurones at risk.

Predicted developments

Research into the causes of Parkinson's disease are likely to show that multiple genetic and environmental factors are involved

Disease of early onset is more likely to be genetic

Modifying the use of drugs already available will improve control of symptoms

New drugs acting on both dopaminergic and non-dopaminergic transmitter systems will become available over the next 10 years

Clinical trials of new drugs with neuroprotective and neurorescue properties are in progress

University Department of Clinical Neurosciences, Royal Free and University College Medical School and Institute of Neurology, University College London, London

A H V Schapira, *professor of clinical neurosciences*

schapira@rfhsm.ac.uk

BMJ 1999;318:311–4

Aetiology and pathogenesis

It is becoming clear that Parkinson's disease is probably not one disease but several with common clinical, pathological, and, possibly, biochemical end points. Although the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the only environmental agent identified so far that is known to be capable of causing parkinsonism (and has done so within 14 days of exposure), other environmental factors such as use of pesticides and herbicides have been linked with an increased risk of disease.

There is increasing evidence for a genetic component in the cause of Parkinson's disease. Several population based studies have found an increased risk (2-3 fold) of developing Parkinson's disease in first degree relatives of a patient.[1] Furthermore, mutations in the α-synuclein gene on chromosome 4[2,3] and the parkin gene on chromosome 6[4] have been identified in families showing autosomal dominant and recessive parkinsonism respectively. These families have somewhat atypical disease—early onset, mild or absent tremor, and, in families with the parkin mutation, no Lewy bodies. A further gene (on chromosome 2), again causing autosomal dominant parkinsonism,[5] is of particular interest as several affected members of the different families identified had features characteristic of idiopathic Parkinson's disease, including age of onset, symptoms, and clinical course. The defect on chromosome 2 seems to have relatively low penetrance (a gene's ability to cause a disease) and might therefore be of more relevance to apparently sporadic disease. Although the α-synuclein mutations have not been identified in sporadic Parkinson's disease, much research is now focused on trying to understand how mutations in different genes can result in specific patterns of neuronal cell death and the clinical features of parkinsonism.

How neurones die in Parkinson's disease

Some biochemical abnormalities have been identified in the affected brain region in Parkinson's disease that provide clues to how genetic or environmental factors may induce cell death. There is much evidence of increased oxidative stress and free radical damage in

the substantia nigra. There is also evidence for a defect of mitochondrial energy production (complex I deficiency).[6] In a group of patients with this mitochondrial deficiency it has been shown that the abnormality was determined by their mitochondrial DNA.[7] Other studies have shown that there may be abnormal calcium handling in dopaminergic neurones and that the gliosis that accompanies nigral cell death may also have a inflammatory component.[8]

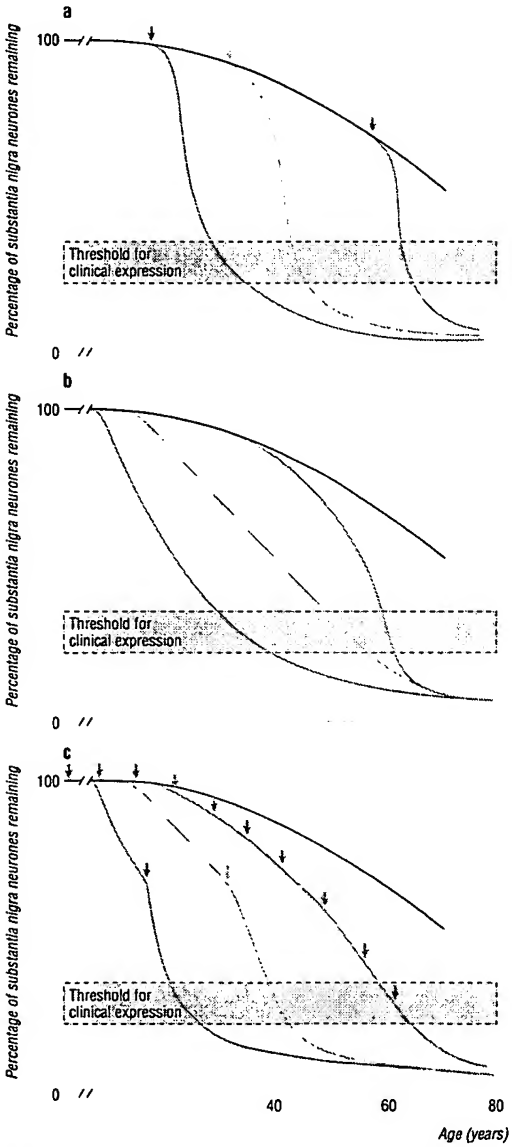


Fig 1 Putative time courses for loss of dopaminergic neurones from substantia nigra relative to different aetiologies of Parkinson's disease. (a) Environmental cause of disease: the environmental insult (arrows) can occur at any time and results in rapid loss of neurones superimposed on age related loss (black line). (b) Genetic cause of disease: the rate of cell death is not known, although patients tend to present at younger age than usual, and rate may vary according to gene defect and patient's genetic background (red, green, and blue lines). (c) Interaction of environmental and genetic causes: genetically induced high rate of cell death (red line) couple with severe point exposure to environmental factor (arrow) results in early presentation; less severe genetic and environmental effects (green line) result in more gradual cell death; and genetic susceptibility superimposed on lifetime exposure to common toxin (blue line) may cause slow cell loss with later presentation of disease

The Lewy bodies found in Parkinson's disease and others, including motor neurone disease, are neuronal intracytoplasmic inclusions. In Parkinson's disease they seem to be collections of protein filaments including ubiquitin and α-synuclein (which is also a component of the amyloid plaques of Alzheimer's disease). This has lead to the suggestion that Parkinson's disease, and possibly other neurodegenerative diseases, may be caused by a fault in intracellular protein degradation that in turn results in protein accumulation. How such a defect in protein handling results in cell death is not known; possibilities include a "black hole" effect of protein attraction, aggregation, clogging of the cytoplasm, and impairment of intracellular function.

Cells may die either by necrosis or apoptosis. Necrosis involves the disintegration of a cell and its organelles and its subsequent removal by phagocytosis through an inflammatory response. Apoptosis is characterised by chromatin condensation, DNA fragmentation, cell shrinkage, relative sparing of organelles, and lack of an inflammatory response. Apoptosis may be programmed, as during embryogenesis, or occur in response to a toxic stimulus. The mitochondrion has recently been shown to have a critical role in the cascade of events that lead to apoptotic cell death.[9] There is now evidence for apoptotic cell death in the brain tissue of patients with Parkinson's disease at the time of death.[10]

This observation may have important implications for developing disease modifying treatment. Apoptotic cell death is relatively rapid. If apoptosis is active at the time of patients' death, it suggests that a proportion of neurones may have been in a pre-apoptotic phase and tipped over into apoptosis by the agonal state. If true, this would offer the opportunity not only to protect nigral neurones but possibly to "rescue" them (fig 2). Many of the biochemical events that precipitate and participate in apoptosis have been defined. Interestingly, both complex I inhibition and oxidative stress (both present in brain tissue affected by Parkinson's disease) may cause apoptotic cell death.

Present treatment options

Drug treatment

With the exception of fetal nigral implants, all treatment currently available for Parkinson's disease is only symptomatic. Because of this, and the potential long term complications of certain drugs, an important principle in treatment is to prescribe drugs only when the symptoms of Parkinson's disease interfere with function to a substantial degree.

Levodopa is the most commonly used treatment for Parkinson's disease. It is always combined with a dopa decarboxylase inhibitor to reduce peripheral side effects and enhance absorption. The development of motor complications with levodopa limits its general usefulness. Direct acting dopamine agonists have been available for some years, but some evidence suggests that those developed more recently have better efficacy and are associated with fewer side effects. Selegiline is a monoamine oxidase B inhibitor and so prolongs the action of dopamine at the synapse. There is evidence

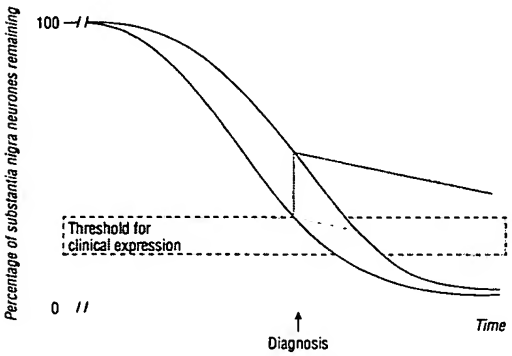


Fig 2 Neurorescue and neuroprotection in Parkinson's disease. Effective neurorescue at diagnosis (red line) will restore damaged neurones that are at risk of death (shaded area between curves) to normal function, and age related loss will probably be attenuated with continuing treatment. Neuroprotection (green line) will prevent further neuronal loss other than by attenuated age related loss

that the early use of selegiline delays a patient's need for additional treatment by 9-12 months. Concerns about the safety of selegiline remain controversial.

The newly developed catechol-*O*-methyltransferase (COMT) inhibitors increase the availability of levodopa to the brain, and their action is complementary to that of the dopa decarboxylase inhibitors. There are concerns about the hepatotoxicity of tolcapone, whereas entacapone, another catechol-*O*-methyltransferase inhibitor, seems safer in this respect. Antimuscarinic drugs and amantadine remain viable alternatives to dopamine related drugs, although their use is often limited by side effects and tolerance.

Surgery

The medical management of Parkinson's disease has its limitations, and new surgical techniques with low morbidity have emerged as a viable alternative for carefully selected patients. Pallidotomy may reduce contralateral dyskinesias and improve bradykinesia and rigidity, and thalamotomy may improve tremor. Deep brain stimulation to the globus pallidus or subthalamic nucleus may substantially improve contralateral symptoms including tremor.[11] Fetal nigral implants improve the symptoms of Parkinson's disease considerably, and postmortem examination of brains of transplanted patients (who had died later of unrelated causes) demonstrated outgrowth and new synaptic formation from the transplanted tissue.[12] Despite its benefits, the application of surgery in treating Parkinson's disease is limited: procedures carry some risk of injury and death, long term effects are unknown, and benefits are only unilateral unless surgery is undertaken on both sides of the brain.

Future treatment prospects

Immediate prospects

The first priority is to maximise the efficacy and safety of the treatments currently available. It has been suggested that levodopa may be toxic and accelerate the death of dopaminergic cells.[13] There is no in vivo evidence to support this, and a recent paper suggests that levodopa might have a trophic effect on dopaminergic neurones.[14] Nevertheless, there is clear evidence that after two to three years of treatment with levodopa, an increasing proportion of patients (about half at five years) begin to experience fluctuations and dyskinesias. This probably relates to the pulsatile stimulation of dopamine receptors that occurs with levodopa and to postsynaptic changes.

The frequency of these complications is substantially less with dopamine agonists, and, at least in animal models, they may not occur if levodopa is not used. Thus, there is a strong argument for starting symptomatic treatment of Parkinson's disease with a dopamine agonist. It is critical that the dose is increased gradually, and many neurologists favour covering the first two weeks of treatment with domperidone, an antiemetic with no extrapyramidal side effects. The newer agonists seem able to control symptoms in a substantial proportion of patients when used alone—for at least up to the first four years of treatment. Levodopa will be required as the disease progresses and symptoms worsen.

Medium term prospects

Preventing or delaying the onset of fluctuations and dyskinesias would be a major advance in treatment, and trials are under way to assess the effectiveness of early monotherapy with a dopamine agonist. A similar study using controlled release levodopa with a catechol-*O*-methyltransferase inhibitor in previously untreated patients would be needed to answer whether a more sustained activation of dopamine receptors results in a lower dyskinesia rate.

Drugs with new forms of action, such as dopamine reuptake inhibitors and adenosine antagonists, have proved promising in animal and early clinical studies. Adenosine A_{2A} receptors are present in high concentration in the striatum—the area to which the dopaminergic neurones of the substantia nigra project. The A_{2A} receptors are localised on neurones containing γ-aminobutyric acid and enkephalin, which also have dopamine receptors. Adenosine A_{2A} stimulation has a negative effect on motor function, whereas antagonists (such as caffeine) can increase locomotor activity, particularly when dopamine receptors are decreased or blocked. Thus, adenosine A_{2A} antagonists may present a new treatment for Parkinson's disease if their efficacy and safety are proved.

Long term prospects

Neuroprotection may be defined as preventing neuronal cell death and maintaining function without necessarily affecting the underlying biochemical mechanisms involved in pathogenesis. At a clinical level, this would mean stopping the progress of the disease. Neurorescue could be considered a mechanism to reverse established metabolic abnormalities and restore normal neuronal function and survival. Clinically, this would result in an improvement in symptoms as well as a halt in the progress of the disease. Inevitably, there will be some overlap between neuroprotection and neurorescue, and their relative benefits will vary according to the stage of disease. The development of such treatments is obviously limited by our knowledge of the biochemical events that cause cell death; at present only a few candidate treatments are available.

Neuroprotection is perhaps best exemplified by strategies designed to prevent cells undergoing apoptosis. Up regulating apoptosis defence genes, such as bcl 2, or down regulating apoptosis promoting genes, such as bax, may be useful if effects can be targeted to nigral neurones. The role of the mitochondrion in the apoptotic pathway is also receiving attention as a possible site at which to direct neuroprotective agents. Cyclosporin A inhibits opening of the mitochondrial megapore, which is associated with loss of membrane potential and the start of apoptotic cell death. Both low dose cyclosporin A and its non-immunosuppressant analogue, *N*-methyl-4-valine cyclosporin, prevent the cell death in vitro induced by toxins that cause parkinsonism.[15] There is also in vitro evidence that selegiline and its desmethyl metabolite have anti-apoptotic properties.[16] However, apoptosis plays an important role in the immune system and in tumour surveillance. Anti-apoptotic treatment for Parkinson's disease will therefore have to be anatomically selective, probably achieved through metabolically targeted delivery systems such as conversion of an inactive precursor to active drug possible only through enzymes of the central nervous system.

Based on our current knowledge of pathogenesis in Parkinson's disease, drugs to prevent or reduce free radical damage or enhance mitochondrial energy production should be of value. Interestingly, there is a reciprocal relation between mitochondrial dysfunction and excess generation of free radicals—the mitochondrion normally produces over 95% of a cell's superoxide ions, and mitochondrial inhibition results in an increased release of these radicals. However, antioxidant treatment has already been attempted with vitamin E without apparent success.[17] Nevertheless, this does not preclude the potential beneficial effects of other antioxidants such as selenium and ubiquinone, or a combination of such drugs. A recent trial has begun with patients using ubiquinone as a means both to increase mitochondrial energy production and decrease free radical release.

Glutamate toxicity is thought to play a role in excitotoxic cell death in Huntington's disease and motor neurone disease, and there is some evidence that this mode of cell death may also be important in Parkinson's disease. This raises the prospect that *N*-methyl-4-valine antagonists or drugs that reduce glutamate release or receptor interaction may be used in Parkinson's disease.

There is some evidence that inflammatory processes may play a role in nerve cell damage in Parkinson's disease, although it is not known whether this is primary or secondary. If this is important in pathogenesis anti-inflammatory drugs or those capable of modulating the immune system (such as non-steroidal anti-inflammatory drugs in Alzheimer's disease and interferon beta in multiple sclerosis) may be worth investigating.

While neuroprotection or neurorescue will be valuable to patients at any stage of disease, treatment will clearly be of most value in those with early disease. The recent advances in the genetics of Parkinson's disease offer the prospect of identifying and treating susceptible individuals before clinical features appear. At first, this may be relevant only to members of those rare families with inherited Parkinson's disease. However, as our knowledge of the genetic component of Parkinson's disease and its relevance to apparently sporadic disease improves, the application of such treatment may be more extensive. Parkinson's disease is unlikely to be caused by genetic factors alone, so identifying possible environmental contributions to aetiology will be important, and their removal or modification will be an essential part of future treatment and prevention.

1 Golbe LI. Genetics of Parkinson's disease. In: Ellenberg JH, Koller WC, Langston KW, eds. *Etiology of Parkinson's disease*. New York: Marcel Dekker, 1995:115-40.
2 Polymeropoulos M, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045-7.
3 Krüger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the α-synuclein in Parkinson's disease. *Nature Genet* 1998;18:106-8.
4 Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Monoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;392:605-8.
5 Gasser T, Müller-Myhsok B, Wszolek Z, Oehlmann R, Calne DB, Bonifati V, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nature Genet* 1998;18:262-5.
6 Schapira AHV, Gu M, Taanman J-W, Tabrizi SJ, Seaton T, Cleeter M, et al. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Ann Neurol* 1998;44(suppl 1):S89-98.
7 Gu M, Cooper JM, Taanman J-W, Schapira AHV. Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Ann Neurol* 1998;44:177-86.
8 Hirsch EC, Hunot S, Damier P, Faucheux. Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration? *Ann Neurol* 1998;44(suppl 1):S115-20.
9 Zamzami N, Hirsch T, Dallaporta B, Petit PX, Kroemer G. Mitochondrial implication in accidental and programmed cell death: apoptosis and necrosis. *J Bioenerg Biomembr* 1997;29:185-93.
10 Tatton NA, Maclean-Fraser A, Tatton WG, Perl DP, Olanow CW. The fluorescent double-labelling method to detect and confirm apoptotic nuclei in Parkinson's disease. *Ann Neurol* 1998;44(suppl 1):S142-8.
11 Limousin P, Krack P, Pollack P, Benazzouz A, Ardouin C, Hoffmann D, et al. Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. *N Engl J Med* 1998;339:1105-11.
12 Olanow CW, Kordower JH, Freeman TB. Fetal nigral transplantation as a therapy for Parkinson's disease. *Trends Neurosci* 1996;19:102-9.
13 Fahn S. Welcome news about levodopa, but uncertainty remains. *Ann Neurol* 1998;43:551-4.
14 Murer MG, Dziewczapolski G, Menalled LB, Garcia MC, Agid Y, Gershanik O, et al. Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery in rats with moderate nigrostrial lesions. *Ann Neurol* 1998;43:561-75.
15 Seaton TA, Cooper JM, Schapira AHV. Free radical scavengers protect dopaminergic cell lines from apoptosis induced by mitochondrial complex I inhibitors. *Brain Res* 1997;777:110-8.
16 Mytilineou C, Radcliffe PM, Olanow CW. L-(-)-desmethylselegiline, a metabolite of selegiline [L-(-)-deprenyl], protects mesencephaliic dopamine neurons from excitotoxicity in vitro. *J Neurochem* 1997; 68:434-6.
17 Parkinson Study Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 1993;328:176-83.

Endpiece

New Year's Eve

Every man has two birth-days: two days at least, in every year, which set him upon revolving the lapse of time, as it affects his mortal duration. The one is that which in an especial manner he termeth *his*. In the gradual desuetude of old observances, this custom of solemnising our proper birth-day hath nearly passed away, or is left to children, who reflect nothing at all about the matter, nor understand anything in it beyond cake and orange. But the birth of a New Year is of an interest too wide to be pretermitted by king or cobbler. No one ever regarded the First of January with indifference. It is that from which all date their time, and count upon what is left. It is the nativity of our common Adam.

Charles Lamb, *The Essays of Elia* (1895)

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- [x] BLACK BORDERS
- [ ] IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- [ ] FADED TEXT OR DRAWING
- [ ] BLURRED OR ILLEGIBLE TEXT OR DRAWING
- [ ] SKEWED/SLANTED IMAGES
- [ ] COLOR OR BLACK AND WHITE PHOTOGRAPHS
- [ ] GRAY SCALE DOCUMENTS
- [ ] LINES OR MARKS ON ORIGINAL DOCUMENT
- [x] REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- [ ] OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.